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(71) Applicant *(for all designated States except US)*: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SCHÜLEIN, Martin [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). ANDERSEN, Lene, Nonboe [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). LASSEN, Søren, Flensted [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). KAUPPINEN, Markus, Sakari [FI/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). LANGE, Lene [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). NIELSEN, Ruby, Ilum [DK/DK]; Novo Nordisk a/s, Novo Allé, DK-2880 Bagsværd (DK). IHARA, Michiko [JP/JP]; Novo Nordisk Bioindustry Ltd., Makuhari Techno Garden CB-6, 3, Nakase 1-chome, Chiba-shi 261-01 (JP). TAKAGI, Shinobu [JP/JP]; Novo Nordisk Bioindustry Ltd., Makuhari Techno Garden CB-6, 3, Nakase 1-chome, Chiba-shi 261-01 (JP).

(74) Common Representative: NOVO NORDISK A/S; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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(57) Abstract

An enzyme preparation consisting essentially of an enzyme having cellulolytic activity and comprising a first amino acid sequence of 14 residues having the sequence Thr Arg X3 X4 Asp Cys Cys X8 X9 X10 Cys X12 Trp X14, in which X3 and X4 independently is Trp, Tyr or Phe; X8 is Arg, Lys or His; each of X9, X10, X12 and X13 is any of the 20 naturally occurring amino acid residues; and a second amino acid sequence of 5 residues having the sequence Trp Cys Cys XX4 Cys, in which XX4 is any of the 20 naturally occurring amino acid residues with the proviso that, in the first amino acid sequence, (i) when X12 is Ser, then X14 is not Ser, and (ii) when X12 is Gly, then X14 is not Ala; performs excellently in detergent, laundering, textile and papermaking pulp applications.

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NOVEL ENDOGLUCANASES

The present invention relates to novel enzyme preparations comprising an enzyme exhibiting endoglucanase activity which performs very good in industrial applications such as laundry compositions, for biopolishing of newly manufactured textiles, for providing an abraded look of cellulosic fabric or garment, and for treatment of paper pulp. Further, the invention relates to DNA constructs encoding such enzymes, a method for providing a gene encoding for such enzymes, a method of producing the enzymes, enzyme preparations containing such enzymes, and the use of these enzymes for a number of industrial applications.

BACKGROUND OF THE INVENTION

Cellulases or cellulytic enzymes are enzymes involved in hydrolyses of cellulose. In the hydrolysis of native cellulose, it is known that there are three major types of cellulase enzymes involved, namely cellobiohydrolase (1,4-β-D-glucan cellobiohydrolase, EC 3.2.1.91), endo-β-1,4-glucanase (endo-1,4-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21).

Cellulases are synthesized by a large number of microorganisms which include fungi, actinomycetes, myxobacteria and true bacteria but also by plants. Especially endoglucanases of a wide variety of specificities have been identified.

A very important industrial use of cellulytic enzymes is the use for treatment of cellulosic textile or fabric, e.g. as ingredients in detergent compositions or fabric

softener compositions, for bio-polishing of new fabric (garment finishing), and for obtaining a "stone-washed" look of cellulose-containing fabric, especially denim, and several methods for such treatment have been suggested, e.g. in GB-A-1 368 599, EP-A-0 307 564 and EP-A-0 435 876, WO 91/17243, WO 91/10732, WO 91/17244, PCT/DK95/000108 and PCT/DK95/00132.

Another important industrial use of cellulytic enzymes is the use for treatment of paper pulp, e.g. for improving the drainage or for deinking of recycled paper.

Especially the endoglucanases (EC No. 3.2.1.4) constitute an interesting group of hydrolases for the mentioned industrial uses. Endoglucanases catalyses endo hydrolysis of 1,4-β-D-glycosidic linkages in cellulose, cellulose derivatives (such as carboxy methyl cellulose and hydroxy ethyl cellulose), lichenin, β-1,4 bonds in mixed β-1,3 glucans such as cereal β-D-glucans or xyloglucans and other plant material containing cellulosic parts. The authorized name is endo-1,4-β-D-glucan 4-glucano hydrolase, but the abbreviated term endoglucanase is used in the present specification. Reference can be made to T.-M. Enveri, "Microbial Cellulases" in W.M. Fogarty, Microbial Enzymes and Biotechnology, Applied Science Publishers, p. 183-224 (1983); Methods in Enzymology, (1988) Vol. 160, p. 200-391 (edited by Wood, W.A. and Kellogg, S.T.); Béguin, P., "Molecular Biology of Cellulose Degradation", Annu. Rev. Microbiol. (1990), Vol. 44, pp. 219-248; Béguin, P. and Aubert, J-P., "The biological degradation of cellulose", FEMS Microbiology Reviews 13 (1994) p.25-58; Henrissat, B., "Cellulases and their interaction with cellulose", Cellulose (1994), Vol. 1, pp. 169-196.

Fungal endoglucanases have been described in numerous publications, especially those derived from species as e.g. *Fusarium oxysporum*, *Trichoderma reesei*, *Trichoderma*

longibrachiatum, *Aspergillus aculeatus*, *Neocallimastix patriciarum*, and e.g. from species of the genera *Piromyces*, *Humicola*, *Myceliophthora*, *Geotricum*, *Penicillium*, *Irpex*, *Coprinus*.

For example, fungal endoglucanases have been described by Sheppard, P.O., et al., "The use of conserved cellulase family-specific sequences to clone Cellulase homologue cDNAs from *Fusarium oxysporum*, Gene, (1994), Vol. 15, pp. 163-167, Saloheimo, A., et al., "A novel, small endoglucanase gene, *egl5*, from *Trichoderma reesei* isolated by expression in yeast", Molecular Microbiology (1994), Vol. 13(2), pp. 219-228; van Arsdell, J.N. et al, (1987), Cloning, characterization, and expression in *Saccharomyces cerevisiae* of endoglucanase I from *Trichoderma reesei*, Bio/Technology 5: 60-64; Penttilä, M. et al., (1986), "Homology between cellulase genes of *Trichoderma reesei*: complete nucleotide sequence of the endoglucanase I gene", *Gene* 45:253-263; Saloheimo, M. et al, (1988), "EGIII, a new endoglucanase from *Trichoderma reesei*: the characterization of both gene and enzyme", *Gene* 63:11-21; Gonzáles, R., et al., "Cloning, sequence analysis and yeast expression of the *egl1* gene from *Trichoderma longibrachiatum*", Appl. Microbiol. Biotechnol., (1992), Vol. 38, pp. 370-375; Ooi, T. et al. "Cloning and sequence analysis of a cDNA for cellulase (FI-CMCase) from *Aspergillus aculeatus*", Curr. Genet., (1990), Vol. 18, pp. 217-222; Ooi, T. et al, "Complete nucleotide sequence of a gene coding for *Aspergillus aculeatus* cellulase (FI-CMCase)", Nucleic Acids Research, (1990), Vol. 18, No. 19, p. 5884; Xue, G. et al., "Cloning and expression of multiple cellulase cDNAs from the anaerobic rumen fungus *Neocallimastix patriciarum* in *E. coli*", J. Gen. Microbiol., (1992), Vol. 138, pp. 1413-1420; Xue, G. et al., "A novel polysaccharide hydrolase cDNA (*celD*) from *Neocallimastix patriciarum* encoding three multi-functional catalytical domains with high

endoglucanase, cellobiohydrolase and xylanase activities", J. Gen. Microbiol., (1992), Vol. 138, pp. 2397-2403; Zhou, L. et al., "Intronless *celB* from the anaerobic fungus *Neocallimastix patriciarum* encodes a modular family A endoglucanase", Biochem. J., (1994), Vol. 297, pp. 359-364; Dalbøge, H. and Heldt-Hansen, H.P., "A novel method for efficient expression cloning of fungal enzyme genes", Mol. Gen. Genet., (1994), Vol. 243, pp. 253-260; Ali, B.R.S. et al., "Cellulases and hemicellulases of the anaerobic fungus *Piromyces* constitute a multiprotein cellulose-binding complex and are encoded by multigene families", FEMS Microbiol. Lett., (1995), Vol. 125, No. 1, pp. 15-21. Further, the DNA Data Bank of Japan (DDBJ database publicly available at Internet) comprises two DNA sequences cloned from *Penicillium janthinellum* encoding endoglucanases (cloned by A. Koch and G. Mernitz, respectively) and a DNA sequence cloned from *Humicola grisea var. thermoidea* encoding an endoglucanase (cloned by T. Uozumi). Two endoglucanases from *Macrophomina phaseolina* have been cloned and sequenced, see Wang, H.Y. and Jones, R.W.: "Cloning, characterization and functional expression of an endoglucanase-encoding gene from the phytopathogenic fungus *Macrophomina phaseolina*" in Gene, 158:125-128, 1995, and Wang, H.Y. and Jones, R.W.: "A unique endoglucanase-encoding gene cloned from the phytopathogenic fungus *Macrophomina phaseolina*" in Applied And Environmental Microbiology, 61:2004-2006, 1995. One of these endoglucanases shows high homology to the egl3 endoglucanase from the fungus *Trichoderma reesei*, the other shows homology to the egl1 from the microbial phytopathogen *Pseudomonas solanacearum* indicating that both endoglucanases belong to family 5 of glycosyl hydrolases (B. Henrissat, Biochem J **280**:309-316 (1991)). Filament-specific expression of a cellulase gene in the dimorphic fungus *Ustilago maydis* is disclosed in Schauwecker, F. et al. (1995).

WO 91/17243 (Novo Nordisk A/S) discloses a cellulase preparation consisting of a homogenous endoglucanase component immunoreactive with an antibody raised against a highly purified 43 kDa endoglucanase derived from *Humicola insolens*, DSM 1800; WO 91/17244 (Novo Nordisk A/S) discloses a new (hemi)cellulose degrading enzyme, such as an endoglucanase, a cellobiohydrolase or a β-glucosidase, which may be derived from fungi other than *Trichoderma* and *Phanerochaete*; WO 93/20193 discloses an endoglucanase derivable from *Aspergillus aculeatus*; WO 94/21801 (Genencor Inc.) concerns a cellulase system isolated from *Trichoderma longibrachiatum* exhibiting endoglucanase activity; WO 94/26880 (Gist Brocades N.V.) discloses an isolated mixture of cellulose degrading enzymes, which preferable are obtained from *Trichoderma*, *Aspergillus* or *Disporotrichum*, comprising endoglucanase, cellobiohydrolase, and xyloglucanase activity; and WO 95/02043 (Novo Nordisk A/S) describes an enzyme with endoglucanase activity derived from *Trichoderma harzianum*, which can be used for a number of purposes including *e.g.* degradation or modification of plant cell walls.

It is also known that cellulases may or may not have a cellulose binding domain (a CBD). The CBD enhances the binding of the enzyme to a cellulose-containing fiber and increases the efficacy of the catalytic active part of the enzyme.

There is an ever existing need for providing novel cellulase enzyme preparations which may be used for applications where cellulase, preferably an endoglucanase, activity is desirable.

The object of the present invention is to provide novel enzyme preparations having substantial cellulytic activity at acid, neutral or alkaline conditions and improved

performance in paper pulp processing, textile treatment, laundry processes or in animal feed; preferably novel cellulases, more preferably well-performing endoglucanases, which are contemplated to be producible or produced by recombinant techniques.

SUMMARY OF THE INVENTION

Surprisingly, it has been found that a group of endoglucanases having certain unique characteristics perform very good in those industrial applications for which endoglucanases are conventionally used. These unique characteristics can be described in terms of conserved regions of the amino acid sequence of the enzyme protein and the inventors have found that cellulytic enzymes, i.e. enzymes exhibiting cellulytic activity, having certain conserved regions are very effective e.g. in the treatment of laundry, in the treatment of newly manufactured textile, in the treatment of papermaking pulp.

Accordingly, in its first aspect the present invention relates to an enzyme preparation consisting essentially of an enzyme having cellulytic activity and comprising a first amino acid sequence consisting of 14 amino acid residues having the following sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp	Xaa
1	2	3	4	5	6	7	8	9	10	11	12	13	14

and a second amino acid sequence consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, 12 and 14, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues with the provisos that, in the first amino acid sequence, (i) when the amino residue in position 12 is Ser, then the amino acid residue in position 14 is not Ser, and (ii) when the amino residue in position 12 is Gly, then the amino acid residue in position 14 is not Ala.

This surprising finding of clearly recognisable conserved regions, in spite of rather prominent variations found within well-performing endoglucanase enzymes, is a result of studies of a number of fungal DNA sequences encoding for specific amino acid sequences of enzymes having significant cellulytic, especially endoglucanase, activities.

Based on this finding, a novel molecular method taylored to screen specifically for genomic DNA or cDNA characterised by encoding the enzymes of the invention has been developed. As tools for this three sets of degenerated primers were constructed. Accordingly, in its second aspect, the invention relates to a method for providing a gene encoding for cellulytic enzymes having the above conserved regions.

By using this method, i.e. the set of primers for a PCR screening on genomic DNA, it was surprisingly found that DNA encoding for said enzymes can be found from a broad range of fungi, belonging to taxonomically very different

organisms and inhabiting ecologically very different niches.

Further, by using this method it has been possible to find DNA sequences encoding for the core regions (catalytically active regions or domains) of said enzymes without any attached cellulose binding domain (CBD) which core regions of enzymes would not have been selected by using conventional performance based screening approaches. The inventors have verified experimentally that the linking of a CBD region to a core region enzyme (comprising the catalytically active region or domain of the enzyme) of the present invention results in a significantly improved performance, e.g. a fifty times higher performance, of the multiple domain enzyme.

Accordingly, the present invention provides novel cellulases, especially endoglucanases, having improved performance in industial applications, either in their native form, or homo- or heterologously produced.

In further aspects, the present invention relates to novel cellulytic enzyme preparations which are derivable from taxonomically specific phyli, classes, orders, families, genera, and species; e.g. from Basidiomycotous *Hymenomycetes*, *Zygomycota*, *Chytridiomycota*; or from the classes *Discomycetes*, *Loculoascomycetes*, *Plectomycetes*; *Archaeascomycetes*, *Hemiascomycetes* or from the orders *Diaportales*, *Xylariales*, *Trichosphaeriales*, *Phyllachorales*; or from the families *Nectriaeae*, *Sordariaceae*, *Chaetomiaceae*, *Ceratostomaceae*, *Lasiosphaeriaceae*; or from the genera *Cylindrocarpon*, *Gliocladium*, *Volutella*, *Scytalidium*, *Acremonium*, or from the species *Fusarium lycopersici*, *Fusarium passiflora*, *Fusarium solani*, *Fusarium anguioides*, *Fusarium poae*, *Humicola nigrescens*, *Humicola grisea*, especially such consisting essentially of an enzyme comprising an amino acid sequence selected

from the group consisting of the sequences

Xaa Thr Arg Xaa Phe Asp Xaa
1 2 3 4 5 6 7 ;

Xaa Thr Arg Xaa Tyr Asp Xaa
1 2 3 4 5 6 7 ; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein, in position 4, Xaa is Trp, Tyr or Phe; and in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

More specifically, the enzyme preparation of the invention is preferably obtainable from the taxonomically specific phyli, classes, orders, families, genera, and species mentioned above which all produce endoglucanases comprising a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein, in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe; in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe; in position 8 of the first sequence, the amino acid is Arg, Lys or His; in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

In yet further aspects, the present invention provides DNA constructs comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises the DNA sequence shown in SEQ ID Nos.

1, 4, 6, 8, 10, 12, 16, and 19, respectively, or analogues thereof.

The present invention also relates to a recombinant expression vector comprising a DNA construct of the invention; to a cell comprising a DNA construct or a recombinant expression vector of the invention; to a method of producing an enzyme, e.g a recombinant enzyme, of the invention; to a method of providing colour clarification of laundry by using the enzyme of the invention; to a laundry composition comprising the enzyme of the invention; to uses of the enzyme of the invention for degradation or modification of plant material, e.g. cell walls, for treatment of fabric, textile or garment, for treatment of paper pulp; and to an enzyme preparation which is enriched in an enzyme of the present invention.

THE DRAWINGS

Figure 1 is an alignment of the deduced encoded amino acid sequences of *Acremonium sp. (I), Volutella colletotrichoides, Crinipellis scabella, Acremonium sp. (II), Myceliophthora thermophila, Thielavia terrestris, Macrophomina phaseolina*. The Pileup program (Feng and Doolittle, 1987) (GCG package, version 8.0) was used to create the best alignment. Identical residues in at least four sequences (boxed) are indicated around the corresponding amino acids.

Figure 2
Figure 2a,b,c illustrates the taxonomic classification within the Fungal Kingdom of all the microorganisms disclosed herein as being capable of producing said enzyme preparations and enzymes of the invention.

The taxonomic classification used herein builds primarily

on the system used in the :NIH Data Base (Entrez, version spring 1996) available on World Wide Web: (http://www3.ncbi.nlm.nih.gov/htbin/ef/entrezTAX). Regarding classification of organisms which are not included in the Entrez data base the following generally available and world wide accepted reference books have been used:

For Ascomycetes: Eriksson, O.E. & Hawksworth, D.L.: Systema Ascomycetum vol 12 (1993).

For Basidiomycetes: Jülich, W.: Higher Taxa of Basidiomycetes, Bibliotheca Mycologia 85, 485pp (1981).

For Zygomycetes: O'Donnell, K.: Zygomycetes in culture, University of Georgia, US, 257pp (1979).

General mycological reference books:

Hawksworth, D.L., Kirk, P.M., Sutton, B.C. and Pegler, D.N.: Dictionary of the fungi, International Mycological Institute, 616pp (1995);

Von Arx, J.A.: The genera of fungi sporulating in culture, 424pp (1981).

The taxonomic implacement of the genus Humicola has untill recently remained unclear. However, studies of 18SRNA of a wide selection of Sordariales has given strong indications of referring Humicola to the order Sordariales (Taylor, Clausen & Oxenbøll, unpublished). Further these data suggests Humicola along with Scytalidium to be only rather distantly related to the families Sordariaceae, Chaetomiaceae, Ceratostomataceae, and Lasiosphaeriaceae. In accordance with the above Humicola and Scytalidium are here placed within the order Sordariales, with unclassified Family.

Figure 3 is an alignment of the deduced partial amino acid sequences derived from a selection of 26 of the 46 microorganisms described in Example 5.

DETAILED DESCRIPTION OF THE INVENTION

In the present context, the term "the 20 naturally occuring amino acid residues" denotes the 20 amino acid residues usually found in proteins and conventionally known as alanine (Ala or A), valine (Val or V), leucine (Leu or L), isoleucine (Ile or I), proline (Pro or P), phenylalanine (Phe or F), tryptophan (Trp or W), methionine (Met or M), glycine (Gly or G), serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), glutamine (Gln or Q), aspartic acid (Asp or D), glutamic acid (Glu or E), lysine (Lys or K), arginine (Arg or R), and histidine (His or H).

According to the present invention there is provided novel well-performing endoglucanases comprising conserved amino acid sequence regions, especially a first amino acid sequence consisting of 14 amino acid residues having the following sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp	Xaa
1	2	3	4	5	6	7	8	9	10	11	12	13	14

and a second amino acid sequence consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein,
in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, 12 and 14, respectively, of the first

sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues with the provisos that, in the first amino acid sequence, (i) when the amino residue in position 12 is Ser, then the amino acid residue in position 14 is not Ser, and (ii) when the amino residue in position 12 is Gly, then the amino acid residue in position 14 is not Ala.

Preferably, the enzyme of the invention is of microbial origin, i.e. obtainable from a microorganism such as a fungus.

In a preferred embodiment, the amino acid residue in position 9 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of proline and threonine.

In another preferred embodiment, the amino acid residue in position 10 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably serine.

In yet another preferred embodiment, the amino acid residue in position 12 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of alanine and glycine.

In yet another preferred embodiment, the amino acid resi-

due in position 14 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine, tryptophan, glutamic acid and aspartic acid, preferably from the group consisting of proline, threonine, serine, alanine, glutamic acid and aspartic acid.

Preferably, the amino acid residue in position 4 of the second sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine, tryptophan, glutamic acid and aspartic acid, more preferably from the group consisting of alanine, glycine, and glutamine.

Examples of more preferred embodiments are such wherein, in the first sequence, the amino acid residue in position 3 is tyrosine; or the amino acid residue in position 4 is tryptophan; or the amino acid residue in position 8 is lysine.

In an especially preferred embodiment, the enzyme of the invention has a first sequence comprising the amino acid sequence

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	,

or the amino acid sequence

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Thr	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	,

or the amino acid sequence

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Gly	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	.

In a second aspect, the present invention provides a method for finding and cloning of such an enzyme which

encoding such an enzyme which method comprises hybridization, e.g. PCR amplification, under standard conditions with an oligonucleotide derived from any of the conserved regions, illustrated in Fig.1.

A useful oligonucleotide comprises a nucleotide sequence encoding at least a pentapeptide comprised in a peptide selected from the group consisting of

a.

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp	Xaa
1	2	3	4	5	6	7	8	9	10	11	12	13	14

the amino acid in position 3 or 4 being Trp, Tyr or Phe;
the amino acid in postion 8 being Arg, Lys or His;
he amino acid in position 9, 10, 12 and 14, respectively, being any of the 20 naturally occurring amino acid residues ; and

b.

Trp	Cys	Cys	Xaa	Cys	Tyr
1	2	3	4	5	6

the amino acid in position 4 being any of the 20 naturally occurring amino acid residues ; and

c.

Xaa	Pro	Gly	Gly	Gly	Xaa	Gly	Xaa	Phe
1	2	3	4	5	6	7	8	9

the amino acid in position 1 being Met or Ile;
the amino acid in position 6 and 8, respectively, being Leu, Ile or Val; and

d.

Gly	Cys	Xaa	Xaa	Arg	Xaa	Asp	Trp	Xaa
1	2	3	4	5	6	7	8	9

the amino acid in position 3 being any of the 20 naturally occurring amino acid residues;
the amino acid in position 4 and 6, respectively, being Trp, Tyr or Phe; and
the amino acid in position 9 being Phe or Met;

The useful oligonucleotides also comprises nucleotide

sequences complementary to the sequences mentioned.

In a preferred embodiment of the method of the invention, the oligonucleotide corresponds to a PCR primer selected from the PCR primers

sense:

5'-$\mathrm{CCCCAAGCTTACI^{A}/_{C}GITA^{C}/_{T}TGGGA^{C}/_{T}TG^{C}/_{T}TG^{C}/_{T}AA^{A}/_{G}{}^{A}/_{C}C}$-3'

antisense 1:

5'- $\mathrm{CTAGTCTAGATA^{A}/_{G}CAIGC^{A}/_{G}CA^{A}/_{G}CACC}$ -3';

antisense 2:

$\mathrm{CTAGTCTAGAAAIA^{A}/_{G}/^{T}ICCIA^{A}/^{C}/^{G}ICCICCICCIGG}$ -3'; and

antisense 3:

5'- $\mathrm{CTAGTCTAGAIAACCA^{A}/_{G}TCA^{A}/_{G}{}^{A}/_{T}AIC^{G}/_{T}CC}$-3.

In a third aspect, the present invention provides an enzyme preparation which essentially consists of an enzyme having cellulytic activity and having the conserved regions found by the inventors, i.e. which comprises a peptide consisting of 7 amino acid residues having the following sequence

Xaa	Thr	Arg	Xaa	Phe	Asp	Xaa	
1	2	3	4	5	6	7	;
Xaa	Thr	Arg	Xaa	Tyr	Asp	Xaa	
1	2	3	4	5	6	7	; and
Xaa	Thr	Arg	Xaa	Trp	Asp	Xaa	
1	2	3	4	5	6	7	

wherein, in position 4, Xaa is Trp, Tyr or Phe; and in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

This enzyme is obtainable from a strain belonging to Basidiomycotous *Hymenomycetes* (see Fig.2), more preferably to the group consisting of the orders *Agaricales, Auriculariales,* and *Aphyllophorales*, even more preferably to the group consisting of the families *Exidiaceae, Tricholomataceae, Coprinaceae, Schizophyllaceae, Bjerkanderaceae* and *Polyporac ae*,

especially to the group consisting of the genera *Exidia, Crinipellis, Fomes, Panaeolus, Trametes, Schizophyllum,* and *Spongipellis*.

Specific examples are endoglucanases obtainable from a strain belonging to the group consisting of the species *Exidia glandulosa, Crinipellis scabella, Fomes fomentarius,* and *Spongipellis sp.*, more specific examples being *Exidia glandulosa,* CBS 277.96, *Crinipellis scabella,* CBS 280.96, *Fomes fomentarius,* CBS 276.96, and *Spongipellis sp.*, CBS 283.96.

Exidia glandulosa was deposited at Centraalbureau voor Schimmelcultures, Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands, on 12 March, 1996, under the deposition number CBS 277.96; *Crinipellis scabella* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 280.96, *Fomes fomentarius* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 276.96, and *Spongipellis sp.* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 283.96; all deposited under the Budapest Treaty.

The enzyme preparation of the invention is also obtainable from a strain belonging to *Chytridiomycota*, preferably from a strain belonging to the class of *Chytridiomycetes*, more preferably belonging to the group consisting of the order *Spizellomycetales* , even more preferably to the family *Spizellomycetaceae*, especially belonging to the genus *Rhizophlyctis*. A specific example is a strain belonging to the species *Rhizophlyctis rosea*, more specifically to *Rhizophlyctis rosea*, CBS 282.96.

Rhizophlyctis rosea was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition

number CBS 282.96; under the Budapest Treaty.

The enzyme preparation of the invention is also obtainable from a strain belonging to *Zygomycota*, preferably belonging to the class *Zygomycetes*, more preferably to the order *Mucorales*, even more preferably to the group of families consisting of *Mucoraceae and Thamnidiaceae*, especially belonging to the group consisting of the genera *Rhizomucor*, *Phycomyces and Chaetostylum*. Specific examples are strains belonging to the genera *Rhizomucor pusillus*, *Phycomyces nitens*, and *Chaetostylum fresenii* more specifically to *Rhizomucor pusillus*, IFO 4578, and *Phycomyces nitens*, IFO 4814 and *Chaetostylum fresenii*, NRRL 2305.

Further, the enzyme preparation of the invention is also obtainable from a strain belonging to the group consisting of *Archaeascomycetes, Discomycetes, Hemiascomycetes, Loculoascomycetes, and Plectomycetes*, preferably belonging to the group consisting of the orders *Pezizales*, *Rhytismatales*, *Dothideales*, and *Eurotiales*. Especially, the enzyme is obtainable from a strain belonging the the group consisting of the families *Cucurbitariaceae, Ascobolaceae, Rhytismataceae,* and *Trichocomaceae*, preferably belonging the the group consisting of the genera *Diplodia*, *Microsphaeropsis*, *Ulospora*, *Macrophomina*, *Ascobolus*, *Saccobolus*, *Penicillium*, and *Thermomyces*. Specific examples are enzymes obtainable from a strain belonging the the group consisting of the species *Diplodia gossypina*, *Microsphaeropsis sp.*, *Ulospora bilgramii*, *Aureobasidium sp.*, *Macrophomina phaseolina*, *Ascobolus stictoides*, *Saccobolus dilutellus*, *Peziza*, *Penicillium verruculosum*, *Penicillium chrysogenum*, and *Thermomyces verrucosus*; more specifically *Diplodia gossypina, CBS 274.96, Ulospora bilgramii, NKBC 1444, Macrophomina phaseolina, CBS 281.96, Saccobolus dilutellus, CBS 275.96, Penicillium*

verruculosum, ATCC 62396, Penicillium chrysogenum, ATCC 9480, and *Thermomyces verrucosus*, CBS 285.96.

Diplodia gossypina was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 274.96, *Macrophomina phaseolina* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 281.96, *Saccobolus dilutellus* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 275.96; *Thermomyces verrucosus* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 285.96; all under the Budapest Treaty.

Yet further, the enzyme is obtainable from a strain belonging to the group consisting of the orders *Diaportales, Xylariales, Trichosphaeriales* and *Phyllachorales,* preferably from a strain belonging to the group consisting of the families *Xylariaceae, Valsaceae,* and *Phyllachoraceae*, more preferably belonging to the genera *Diaporthe, Colletotrichum, Nigrospora, Xylaria, Nodulisporum* and *Poronia*. Specific examples are the species *Diaporthe syngenesia, Colletotrichum lagenarium, Xylaria hypoxylon, Nigrospora sp., Nodulisporum sp.,* and *Poronia punctata,* more specifically *Diaporthe syngenesia, CBS 278.96, Colletotrichum lagenarium, ATCC 52609, Nigrospora sp., CBS 272.96, Xylaria hypoxylon, CBS 284.96.*

Diaporthe syngenesia was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 278.96, *Nigrospora sp.* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 272.96, *Xylaria hypoxylon* was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 284.96;

all under the Budapest Treaty.

The enzyme is also obtainable from the unidentified fungal, mitosporic, coleomycetous deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition numbers CBS 270.96, CBS 271.96 and CBS 273.96, respectively, under the Budapest Treaty.

The enzyme is also obtainable from a strain belonging to the group consisting of the genera *Cylindrocarpon*, *Gliocladium*, *Nectria*, *Volutella*, *Sordaria*, *Scytalidium*, *Thielavia*, *Syspastospora*, *Cladorrhinum*, *Chaetomium*, *Myceliphthora* and *Acremonium*, especially from a strain belonging to the group consisting of the species *Cylindrocarpon sp.*, *Nectria pinea*, *Volutella colletotrichoides*, *Sordaria fimicola*, *Sordaria macrospora*, *Thielavia terrestris*, *Thielavia thermophila*, *Syspastospora boninensis*, *Cladorrhinum foecundissimum*, *Chaetomium murorum*, *Chaetomium virescens*, *Chaetomium brasiliensis*, *Chaetomium cunicolorum*, *Myceliophthora thermophila*, *Gliocladium catenulatum*, *Scytalidium thermophila*, and *Acremonium sp.*, more specifically from *Nectria pinea*, *CBS 279.96*, *Volutella colletotrichoides*, *CBS 400.58*, *Sordaria fimicola*, *ATCC 52644*, *Sordaria macrospora*, *ATCC 60255*, *Thielavia terrestris*, *NRRL 8126*, *Thielavia thermophila*, *CCBS 174.70*, *Chaetomium murorum*, *CBS 163.52*, *Chaetomium virescens*, *CBS 547.75*, *Chaetomium brasiliensis*, *CBS 122.65*, *Chaetomium cunicolorum*, *CBS 799.83*, *Syspastospora boninensis*, *NKBC 1515*, *Cladorrhinum foecundissimum*, ATCC 62373, *Myceliophthora thermophila*, CBS 117.65, *Scytalidium thermophila*, ATCC 28085, Gliocladium catenulatum, ATCC 10523, and *Acremonium sp.*, CBS 478.94.

Nectria pinea was deposited at Centraalbureau voor Schimmelcultures on 12 March, 1996, under the deposition number CBS 279.96, and *Acremonium sp.* was deposited in

28 September 1994 under the deposition number CBS 478.94, both according to the Budapest Treaty.

The enzyme is also obtainable from a strain belonging to the group consisting of the species *Fusarium solani, Fusarium anguioides, Fusarium poae, Fusarium oxysporum ssp. lycopersici, Fusarium oxysporum ssp. passiflora, Humicola nigrescens* and *Humicola grisea*, especially *Fusarium oxysporum ssp lycopersici, CBS 645.78, Fusarium oxysporum ssp passiflora, CBS 744.79, Fusarium solani, IMI 107.511, Fusarium anguioides, IFO 4467, Fusarium poae, ATCC 60883, Humicola nigrescens*, CBS 819.73 and *Humicola grisea*, ATCC 22726. It is to be noted that *Humicola grisea* is different from *Humicola grisea var. thermoidea*.

In a preferred embodiment, the enzyme preparation of the invention is derived from the disclosed classes, orders, families, genera and species and essentially consists of an enzyme comprising a first peptide consisting of 13 amino acid residues having the following sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp
1	2	3	4	5	6	7	8	9	10	11	12	13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein, in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe; in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe; in position 8 of the first sequence, the amino acid is Arg, Lys or His; in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence,

the amino acid is any of the 20 naturally occurring amino acid residues.

Preferably, the amino acid residue in position 9 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, more preferably from the group consisting of proline and threonine; the amino acid residue in position 10 of the first sequence which is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably serine; the amino acid residue in position 12 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of alanine and glycine; and the amino acid residue in position 4 of the second sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine, tryptophan, glutamic acid and aspartic acid, more preferably from the group consisting of alanine, glycine, and glutamine.

In further aspects, the present invention provides a DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia*

coli, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively; or

b) an analogue of the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively, which

i) is homologous with the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively,

ii) hybridizes with the same oligonucleotide probe as the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively,

iii) encodes a polypeptide which is homologous with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 1, 4, 6, 8, 10, 12, 16, or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Esch richia coli*,

DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively.

Escherichia coli DSM 10512 was deposited under the Budapest Treaty on 2 February, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10511 was deposited under the Budapest Treaty on 2 February, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10571 was deposited under the Budapest Treaty on 6 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10576 was deposited under the Budapest Treaty on 12 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10583 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10584 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10585 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10586 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10587 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Escherichia coli DSM 10588 was deposited under the Budapest Treaty on 13 March, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Saccharomyces cerevisiae DSM 9770 was deposited under the Budapest Treaty on 24 February, 1995, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Saccharomyces cerevisiae DSM 10082 was deposited under the Budapest Treaty on 30 June, 1995, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Saccharomyces cerevisiae DSM 10080 was deposited under the Budapest Treaty on 30 June, 1995, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

Saccharomyces cerevisiae DSM 10081 was deposited under the Budapest Treaty on 30 June, 1995, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany).

The DNA construct of the invention relating to SEQ ID No. 1 can be isolated from or produced on the basis of a DNA

library of a strain of *Myceliophthora,* in particular a strain of *M. thermophila*, especially *M. thermophila*, CBS 117.65.

The DNA constructs of the invention relating to SEQ ID Nos. 4 and 6 can be isolated from or produced on the basis of a DNA library of a strain of *Acremonium,* especially *Acremonium sp.*, CBS 478.94.

The DNA construct of the invention relating to SEQ ID No. 8 can be isolated from or produced on the basis of a DNA library of a strain of *Thielavia* in particular a strain of *Thielavia terrestris*, especially *Thielavia terrestris,* NRRL 8126.

The DNA construct of the invention relating to SEQ ID No. 10 can be isolated from or produced on the basis of a DNA library of a strain of *Macrophomina,* in particular a strain of *M. phaseolina,* especially *M.phaseolina*, CBS 281.96.

The DNA construct of the invention relating to SEQ ID No. 12 can be isolated from or produced on the basis of a DNA library of a strain of *Crinipellis,* in particular a strain of *C. scabella*, especially *C.scabella*, CBS 280.96.

The DNA construct of the invention relating to SEQ ID No. 19 can be isolated from or produced on the basis of a DNA library of a strain of *Sordaria,* in particular a strain of *Sordaria fimicola*.

In the present context, the "analogue" of the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16 or 19, respectively, is intended to indicate any DNA sequence encoding an enzyme exhibiting endoglucanase activity, which has any or all of the properties i)-iv).

The analogous DNA sequence

a) may be isolated from another or related (e.g. the same) organism producing the enzyme with endoglucanase activity on the basis of the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16 or 19, respectively, e.g. using the procedures described herein; the homologue may be an allelic variant of the DNA sequence comprising the DNA sequences shown herein, i.e. an alternative form of a gene that arises through mutation; mutations can be silent (no change in the encoded enzyme) or may encode enzymes having altered amino acid sequence; the homologue of the present DNA sequence may also be a genus or species homologue, i.e. encoding an enzyme with a similar activity derived from another species,

b) may be constructed on the basis of the DNA sequences shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16 or 19, respectively, e.g. by introduction of nucleotide substitutions which do not give rise to another amino acid sequence of the endoglucanase encoded by the DNA sequence, but which correspond to the codon usage of the host organism intended for production of the enzyme, or by introduction of nucleotide substitutions which may give rise to a different amino acid sequence. However, in the latter case amino acid changes are preferably of a minor nature, that is conservative amino acid substitutions that do not significantly affect the folding or activity of the protein, small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain. See in general Ford et al., Protein Expression and Purification 2: 95-107, 1991. Examples of conservative substitutions are within the

group of basic amino acids (such as arginine, lysine, histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine, isoleucine, valine), aromatic amino acids (such as phenylalanine, tryptophan, tyrosine) and small amino acids (such as glycine, alanine, serine, threonine, methionine).

It will be apparent to persons skilled in the art that such substitutions can be made outside the regions critical to the function of the molecule and still result in an active polypeptide. Amino acids essential to the activity of the polypeptide encoded by the DNA construct of the invention, and therefore preferably not subject to substitution, may be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244, 1081-1085, 1989). In the latter technique mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological (i.e. endoglucanase) activity to identify amino acid residues that are critical to the activity of the molecule. Sites of substrate-enzyme interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labeling. See, for example, de Vos et al., Science 255: 306-312, 1992; Smith et al., J. Mol. Biol. 224: 899-904, 1992; Wlodaver et al., FEBS Lett. 309: 59-64, 1992.

The endoglucanase encoded by the DNA sequence of the DNA construct of the invention may comprise a cellulose binding domain (CBD) existing as an integral part of the encoded enzyme, or a CBD from another origin may be introduced into the endoglucanase enzyme thus creating an enzyme hybride. In this context, the term "cellulose-

binding domain" is intended to be understood as defined by Peter Tomme et al. "Cellulose-Binding Domains: Classification and Properties" in "Enzymatic Degradation of Insoluble Carbohydrates", John N. Saddler and Michael H. Penner (Eds.), ACS Symposium Series, No. 618, 1996. This definition classifies more than 120 cellulose-binding domains (CBDs) into 10 families (I-X), and it demonstrates that CBDs are found in various enzymes such as cellulases, xylanases, mannanases, arabinofuranosidases, acetyl esterases and chitinases. CBDs have also been found in algae, e.g., the red alga Porphyra purpurea as a non-hydrolytic polysaccharide-binding protein, for reference see Peter Tomme et al., supra. However, most of the CBDs are from cellulases and xylanases. CBDs are found at the N or C termini of proteins or are internal. Enzyme hybrids are known in the art, see e.g. WO 90/00609 and WO 95/16782, and may be prepared by transforming into a host cell a DNA construct comprising at least a fragment of DNA encoding the cellulose-binding domain ligated, with or without a linker, to a DNA sequence encoding the enzyme of interest and growing the host cell to express the fused gene. Enzyme hybrids may be described by the following formula:

CBD - MR - X,

wherein CBD is the N-terminal or the C-terminal region of an amino acid sequence corresponding to at least the cellulose-binding domain; MR is the middle region (the linker), and may be a bond, or a short linking group preferably of from about 2 to about 100 carbon atoms, more preferably of from 2 to 40 carbon atoms; or is preferably from about 2 to about 100 amino acids, more preferably of from 2 to 40 amino acids; and X is an N-terminal or C-terminal region of a polypeptide encoded by the DNA sequence of the invention.

The homology referred to in i) above is determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second. The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Needleman, S.B. and Wunsch, C.D., *Journal of Molecular Biology*, 48: 443-453, 1970). Using GAP with the following settings for DNA sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the DNA sequence exhibits a degree of identity preferably of at least 60%, more preferably at least 65%, more preferably at least 70%, even more preferably at least 80%, especially at least 90%, with the coding region of the DNA sequence shown in SEQ ID No.1, 4, 6, 8, 10, 12, or 16, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, or DSM 10576, respectively.

The hybridization referred to in ii) above is intended to indicate that the analogous DNA sequence hybridizes to the same probe as the DNA sequence encoding the endoglucanase enzyme under certain specified conditions which are described in detail in the Materials and Methods section hereinafter. The oligonucleotide probe to be used is the DNA sequence corresponding to the endoglucanase encoding part of the DNA sequence shown in SEQ ID NO 1, 4, 6, 8, 10, 12, or 16 respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively.

The homology referred to in iii) above is determined as the degree of identity between the two sequences indicating a derivation of the first sequence from the second.

The homology may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package (Needleman, S.B. and Wunsch, C.D., *Journal of Molecular Biology*, 48: 443-453, 1970). Using GAP with the following settings for polypeptide sequence comparison: GAP creation penalty of 3.0 and GAP extension penalty of 0.1, the polypeptide encoded by an analogous DNA sequence exhibits a degree of identity preferably of at least 55%, more preferably at least 60%, more preferably at least 65%, even more preferably at least 70%, more preferably at least 80%, especially at least 90%, with the enzyme encoded by a DNA construct comprising the DNA sequence shown in SEQ ID No.1, 4, 6, 8, 10, 12, 16 or 19, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively.

In connection with property iv) above it is intended to indicate an endoglucanase encoded by a DNA sequence isolated from strain *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively, and produced in a host organism transformed with said DNA sequence or the corresponding endoglucanase naturally produced by *Myceliophthora thermophila, Acremonium sp., Thielavia terrestris, Macrophomina phaseolina, Crinipellis scabella, Volutella colletotrichoides*, or *Sordaria fimicola*, respectively. The immunological reactivity may be determined by the method described in the Materials and Methods section below.

In further aspects the invention relates to an expression vector harbouring a DNA construct of the invention, a cell comprising the DNA construct or expression vector and a method of producing an enzyme exhibiting

endoglucanase activity which method comprises culturing said cell under conditions permitting the production of the enzyme, and recovering the enzyme from the culture.

In a still further aspect the invention relates to an enzyme exhibiting endoglucanase activity, which enzyme
a) is encoded by a DNA construct of the invention
b) produced by the method of the invention, and/or
c) is immunologically reactive with an antibody raised against a purified endoglucanase encoded by the DNA sequence shown in SEQ ID No.1, 4, 6, 8, 10, 12, or 16, respectively, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively.

The endoglucanase mentioned in c) above may be encoded by the DNA sequence isolated from the strain *Saccharomyces cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively, and produced in a host organism transformed with said DNA sequence or the corresponding endoglucanase naturally produced by *Myceliophthora thermophila*, *Acremonium sp.*, *Thielavia terrestris*, *Macrophomina phaseolina*, *Crinipellis scabella*, *Volutella colletotrichoides* or *Sordaria fimicola*, respectively.

Generally, in the present context the term "enzyme" is understood to include a mature protein or a precursor form thereof as well to a functional fragment thereof which essentially has the activity of the full-length enzyme. Furthermore, the term "enzyme" is intended to include homologues of said enzyme.

Homologues of the present enzyme may have one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conser-

vative amino acid substitutions that do not significantly affect the folding or activity of the protein, small deletions, typically of one to about 30 amino acids; small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or a small extension that facilitates purification, such as a poly-histidine tract, an antigenic epitope or a binding domain. See in general Ford et al. , Protein Expression and Purification 2: 95-107, 1991. Examples of conservative substitutions are within the group of basic amino acids (such as arginine, lysine, histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine, isoleucine, valine), aromatic amino acids (such as phenylalanine, tryptophan, tyrosine) and small amino acids (such as glycine, alanine, serine, threonine, methionine).

It will be apparent to persons skilled in the art that such substitutions can be made outside the regions critical to the function of the molecule and still result in an active enzyme. Amino acids essential to the activity of the enzyme of the invention, and therefore preferably not subject to substitution, may be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham, 1989). In the latter technique mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for cellulytic activity to identify amino acid residues that are critical to the activity of the molecule. Sites of ligand-receptor interaction can also be determined by analysis of crystal structure as determined by such techniques as nuclear magnetic resonance, crystallography or photoaffinity labelling. See, for example, de Vos et al., 1992; Smith et al., 1992, Wlodaver et al., 1992.

The homologue may be an allelic variant, i.e. an alternative form of a gene that arises through mutation, or an altered enzyme encoded by the mutated gene, but having substantially the same activity as the enzyme of the invention. Hence mutations can be silent (no change in the encoded enzyme) or may encode enzymes having altered amino acid sequence.

The homologue of the present enzyme may also be a genus or species homologue, i.e. an enzyme with a similar activity derived from another species.

A homologue of the enzyme may be isolated by using the procedures described herein.

Molecular screening and cloning by polymerase chain reaction (PCR)

Molecular screening for DNA sequences of the invention may be carried out by polymerase chain reaction (PCR) using genomic DNA or double-stranded cDNA isolated from a suitable source, such as any of the herein mentioned organisms, and synthetic oligonucleotide primers prepared on the basis of the DNA sequences or the amino acid sequences disclosed herein. For instance, suitable oligonucleotide primers may be the primers described in the Materials and Methods section.

In accordance with well-known procedures, the PCR fragment generated in the molecular screening may be isolated and subcloned into a suitable vector. The PCR fragment may be used for screening DNA libraries by e.g. colony or plaque hybridization.

Expression cloning in yeast

The DNA sequence of the invention encoding an enzyme exhibiting endoglucanase activity may be isolated by a general method involving

- cloning, in suitable vectors, a DNA library from a suitable source, such as any of the herein mentioned organisms
- transforming suitable yeast host cells with said vectors,
- culturing the host cells under suitable conditions to express any enzyme of interest encoded by a clone in the DNA library,
- screening for positive clones by determining any endoglucanase activity of the enzyme produced by such clones, and
- isolating the enzyme encoding DNA from such clones.

The general method is further disclosed in WO 94/14953 the contents of which are hereby incorporated by reference. A more detailed description of the screening method is given in Example 1 below.

The DNA sequence coding for the enzyme may for instance be isolated by screening a cDNA library of *Macrophomina phaseolina, Crinipellis scabella, Sordaria fimicola* or *Volutella colletotrichoides*, and selecting for clones expressing the appropriate enzyme activity (i.e. endoglucanase activity) or from *Escherichia coli* DSM 10512 deposited under the Budapest Treaty on 2 February, 1996, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany), or from *Escherichia coli* DSM 10511 deposited under the Budapest Treaty on 2 February, 1996, at DSM, or from Escherichia coli DSM 10576, deposited under the Budapest Treaty on 12 March, 1996, at

DSM; or from *Escherichia coli* DSM 10571 deposited under the Budapest Treaty on 6 March, 1996, at DSM; or by screening a cDNA library of *Myceliphthora thermophila*, CBS 117.65, *Acremonium sp.*, CBS 478.94, or *Thielavia terrestris*, NRRL 8126, and selecting for clones expressing the appropriate enzyme activity (i.e. endoglucanase activity) or from *Saccharomyces cerevisiae* DSM 9770 deposited under the Budapest Treaty on 24 February, 1995, at DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 16, D-38124 Braunschweig, Germany), or from *Saccharomyces cerevisiae* DSM 10082 deposited under the Budapest Treaty on 30 June, 1995, at DSM, from *Saccharomyces cerevisiae* DSM 10080 deposited under the Budapest Treaty on 30 June, 1995, or from *Saccharomyces cerevisiae* DSM 10081 deposited under the Budapest Treaty on 30 June, 1995, at DSM. The appropriate DNA sequence may then be isolated from the clone by standard procedures, e.g. as described in Example 1.

Nucleic acid construct

As used herein the term "nucleic acid construct" is intended to indicate any nucleic acid molecule of cDNA, genomic DNA, synthetic DNA or RNA origin. The term "construct" is intended to indicate a nucleic acid segment which may be single- or double-stranded, and which may be based on a complete or partial naturally occurring nucleotide sequence encoding an enzyme of interest. The construct may optionally contain other nucleic acid segments.

The nucleic acid construct encoding the enzyme of the invention may suitably be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of

the enzyme by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., 1989).

The nucleic acid construct encoding the enzyme may also be prepared synthetically by established standard methods, e.g. the phosphoamidite method described by Beaucage and Caruthers, (1981), or the method described by Matthes et al., (1984). According to the phosphoamidite method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

Furthermore, the nucleic acid construct may be of mixed synthetic and genomic, mixed synthetic and cDNA or mixed genomic and cDNA origin prepared by ligating fragments of synthetic, genomic or cDNA origin (as appropriate), the fragments corresponding to various parts of the entire nucleic acid construct, in accordance with standard techniques.

The nucleic acid construct may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., (1988).

The nucleic acid construct is preferably a DNA construct which term will be used exclusively in this specification and claims.

Recombinant vector

A recombinant vector comprising a DNA construct encoding the enzyme of the invention may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host

cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

The vector is preferably an expression vector in which the DNA sequence encoding the enzyme of the invention is operably linked to additional segments required for transcription of the DNA. In general, the expression vector is derived from plasmid or viral DNA, or may contain elements of both. The term, "operably linked" indicates that the segments are arranged so that they function in concert for their intended purposes, e.g. transcription initiates in a promoter and proceeds through the DNA sequence coding for the enzyme.

The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell.

Examples of suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., J. Biol. Chem. 255 (1980), 12073 - 12080; Alber and Kawasaki, J. Mol. Appl. Gen. 1 (1982), 419 - 434) or alcohol dehydrogenase genes (Young et al., in Genetic Engineering of Microorganisms for Chemicals (Hollaender et al, eds.), Plenum Press, New York, 1982), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., Nature 304 (1983), 652 - 654) promoters.

Examples of suitable promot rs for use in filamentous

fungus host cells are, for instance, the ADH3 promoter (McKnight et al., The EMBO J. 4 (1985), 2093 - 2099) or the tpiA promoter. Examples of other useful promoters are those derived from the gene encoding *A. oryzae* TAKA amylase, *Rhizomucor miehei* aspartic proteinase, *A. niger* neutral α-amylase, *A. niger* acid stable α-amylase, *A. niger* or *A. awamori* glucoamylase (gluA), *Rhizomucor miehei* lipase, *A. oryzae* alkaline protease, *A. oryzae* triose phosphate isomerase or *A. nidulans* acetamidase. Preferred are the TAKA-amylase and gluA promoters.

Examples of suitable promoters for use in bacterial host cells include the promoter of the *Bacillus stearothermophilus* maltogenic amylase gene, the *Bacillus licheniformis* alpha-amylase gene, the *Bacillus amyloliquefaciens* BAN amylase gene, the *Bacillus subtilis* alkaline protease gen, or the *Bacillus pumilus* xylosidase gene, or by the phage Lambda P_R or P_L promoters or the E. coli lac, trp or tac promoters.

The DNA sequence encoding the enzyme of the invention may also, if necessary, be operably connected to a suitable terminator.

The recombinant vector of the invention may further comprise a DNA sequence enabling the vector to replicate in the host cell in question.

The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or the *Schizosaccharomyces pombe* TPI gene (described by P.R. Russell, Gene 40, 1985, pp. 125-130). For filamentous fungi, selectable markers include amdS, pyrG, argB, niaD, sC.

To direct an enzyme of the present invention into the

secretory pathway of the host cells, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) may be provided in the recombinant vector. The secretory signal sequence is joined to the DNA sequence encoding the enzyme in the correct reading frame. Secretory signal sequences are commonly positioned 5′ to the DNA sequence encoding the enzyme. The secretory signal sequence may be that normally associated with the enzyme or may be from a gene encoding another secreted protein.

For secretion from yeast cells, the secretory signal sequence may encode any signal peptide which ensures efficient direction of the expressed enzyme into the secretory pathway of the cell. The signal peptide may be naturally occurring signal peptide, or a functional part thereof, or it may be a synthetic peptide. Suitable signal peptides have been found to be the α-factor signal peptide (cf. US 4,870,008), the signal peptide of mouse salivary amylase (cf. O. Hagenbuchle et al., Nature 289, 1981, pp. 643-646), a modified carboxypeptidase signal peptide (cf. L.A. Valls et al., Cell 48, 1987, pp. 887-897), the yeast BAR1 signal peptide (cf. WO 87/02670), or the yeast aspartic protease 3 (YAP3) signal peptide (cf. M. Egel-Mitani et al., Yeast 6, 1990, pp. 127-137).

For efficient secretion in yeast, a sequence encoding a leader peptide may also be inserted downstream of the signal sequence and upstream of the DNA sequence encoding the enzyme. The function of the leader peptide is to allow the expressed enzyme to be directed from the endoplasmic reticulum to the Golgi apparatus and further to a secretory vesicle for secretion into the culture medium (i.e. exportation of the enzyme across the cell wall or at least through the cellular membrane into the periplasmic space of the yeast cell). The leader peptide may be the yeast α-factor leader (the use of which is

described in e.g. US 4,546,082, EP 16 201, EP 123 294, EP 123 544 and EP 163 529). Alternatively, the leader peptide may be a synthetic leader peptide, which is to say a leader peptide not found in nature. Synthetic leader peptides may, for instance, be constructed as described in WO 89/02463 or WO 92/11378.

For use in filamentous fungi, the signal peptide may conveniently be derived from a gene encoding an *Aspergillus* sp. amylase or glucoamylase, a gene encoding a *Rhizomucor miehei* lipase or protease, a *Humicola lanuginosa* lipase. The signal peptide is preferably derived from a gene encoding *A. oryzae* TAKA amylase, *A. niger* neutral α-amylase, *A. niger* acid-stable amylase, or *A. niger* glucoamylase.

The procedures used to ligate the DNA sequences coding for the present enzyme, the promoter and optionally the terminator and/or secretory signal sequence, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

Host cells

The DNA sequence encoding the present enzyme introduced into the host cell may be either homologous or heterologous to the host in question. If homologous to the host cell, i.e. produced by the host cell in nature, it will typically be operably connected to another promoter sequence or, if applicable, another secretory signal sequence and/or terminator sequence than in its natural environment. The term "homologous" is intended to include a cDNA sequence encoding an enzyme native to the host organism in question. The term "heterologous" is

intended to include a DNA sequence not expressed by the host cell in nature. Thus, the DNA sequence may be from another organism, or it may be a synthetic sequence.

The host cell into which the DNA construct or the recombinant vector of the invention is introduced may be any cell which is capable of producing the present enzyme and includes bacteria, yeast, fungi and higher eukaryotic cells.

Examples of bacterial host cells which, on cultivation, are capable of producing the enzyme of the invention are gram-positive bacteria such as strains of *Bacillus*, such as strains of *B. subtilis, B. licheniformis, B. lentus, B. brevis, B. stearothermophilus, B. alkalophilus, B. amyloliquefaciens, B. coagulans, B. circulans, B. lautus, B. megatherium* or *B. thuringiensis*, or strains of *Streptomyces*, such as *S. lividans* or *S. murinus*, or gram-negative bacteria such as *Echerichia coli*. The transformation of the bacteria may be effected by protoplast transformation or by using competent cells in a manner known per se (cf. Sambrook et al., supra).

When expressing the enzyme in bacteria such as *E. coli*, the enzyme may be retained in the cytoplasm, typically as insoluble granules (known as inclusion bodies), or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed and the granules are recovered and denatured after which the enzyme is refolded by diluting the denaturing agent. In the latter case, the enzyme may be recovered from the periplasmic space by disrupting the cells, e.g. by sonication or osmotic shock, to release the contents of the periplasmic space and recovering the enzyme.

Examples of suitable yeasts cells include cells of *Saccharomyces* spp. or *Schizosaccharomyces* spp., in

particular strains of *Saccharomyces cerevisiae* or *Saccharomyces kluyveri*. Methods for transforming yeast cells with heterologous DNA and producing heterologous enzymes therefrom are described, e.g. in US 4,599,311, US 4,931,373, US 4,870,008, 5,037,743, and US 4,845,075, all of which are hereby incorporated by reference. Transformed cells are selected by a phenotype determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient, e.g. leucine. A preferred vector for use in yeast is the POT1 vector disclosed in US 4,931,373. The DNA sequence encoding the enzyme of the invention may be preceded by a signal sequence and optionally a leader sequence , e.g. as described above. Further examples of suitable yeast cells are strains of *Kluyveromyces*, such as *K. lactis*, *Hansenula*, e.g. *H. polymorpha*, or *Pichia*, e.g. *P. pastoris* (cf. Gleeson et al., J. Gen. Microbiol. 132, 1986, pp. 3459-3465; US 4,882,279).

Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus* spp., *Neurospora* spp., *Fusarium* spp. or *Trichoderma* spp., in particular strains of *A. oryzae*, *A. nidulans*, *A. niger*, or *Fusarium graminearum*. The use of *Aspergillus* spp. for the expression of proteins is described in, e.g., EP 272 277, EP 230 023. The transformation of *F. oxysporum* may, for instance, be carried out as described by Malardier et al., 1989, Gene 78: 147-156.

When a filamentous fungus is used as the host cell, it may be transformed with the DNA construct of the invention, conveniently by integrating the DNA construct in the host chromosome to obtain a recombinant host cell. This integration is generally considered to be an advantage as the DNA sequence is more likely to be stably maintained in the cell. Integration of the DNA constructs into the host chromosome may be performed

according to conventional methods, e.g. by homologous or heterologous recombination.

The transformed or transfected host cell described above is then cultured in a suitable nutrient medium under conditions permitting the expression of the present enzyme, after which the resulting enzyme is recovered from the culture.
The medium used to culture the cells may be any conventional medium suitable for growing the host cells, such as minimal or complex media containing appropriate supplements. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection). The enzyme produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. ion exchange chromatography, gel filtration chromatography, affinity chromatography, or the like, dependent on the type of enzyme in question.

In a still further aspect, the present invention relates to a method of producing an enzyme according to the invention, wherein a suitable host cell transformed with a DNA sequence encoding the enzyme is cultured under conditions permitting the production of the enzyme, and the resulting enzyme is recovered from the culture.

Enzyme Screening driven by taxonomy as well as ecology:

A powerful tool like the molecular screening disclosed herein, designed to detect and select said type of

interesting enzymes, can still not stand on its own. In order to maximize the chances of making interesting discoveries the molecular screening approach was in the present investigation combined with careful selection of which fungi to screen. The selection was done through a thorough insight in the identification of fungi, in taxonomical classification and in phylogenetic relationships.

A taxonomic hot spot for production of cellulytic enzymes can further only be fully explored if also the ecological approach is included. Thorough knowledge about the adaptation to various substrates (especially saprotrophic, necrotrophic or biotrophic degradation of plant materials) are prerequisites for designing an intelligent screening and for managing a successful selection of strains and ecological niches to be searc-hed.

Both the taxonomy and the ecological approach disclosed herein aim at maximizing discovery of said enzymes in the molecular screening program. However, still several hundreds (or if all preliminary work is included) several thousand fungi have been brought in culture in order to detect the 53 hits of said type of cellulytic enzyme here reported.

The screening and cloning may be carried out using the following:

MATERIALS AND METHODS

List of organisms:

Saccharomyces cerevisiae, DSM 9770, DSM 10082, DSM 10080, DSM 10081, or *Escherichia coli*, DSM 10512, DSM 10511, DSM 10571, DSM 10576, respectively, containing the plasmid

comprising the full length DNA sequence, coding for the endoglucanase of the invention, in the shuttle vector pYES 2.0.

Escherichia coli DSM 10583, 10584, 10585, 10586, 10587, and 10588.

Diplodia gossypina Cooke
Deposit of Strain, Acc No: CBS 274.96
Classification: Ascomycota, Loculoascomycetes, Dothideales, Cucurbitariaceae

Ulospora bilgramii (Hawksw. et al.) Hawksw. et al.
Acc No of strain: NKBC 1444, Nippon University, (Prof. Tubaki collection)
Classification: Ascomycota, Loculoascomycetes, Dothideales, (family unclassified)

Microsphaeropsis sp.
Isolated from: Leaf of Camellia japonica (Theaceae, Guttiferales), grown in Kunming Botanical garden, Yunnan Province, China
Classification: Ascomycota, Loculoascomycetes, Dothideales, (family unclassified)

Macrophomina phaseolina (Tassi) Goidannich
Syn: Rhizoctonia bataticola
Deposit of Strain, Acc No.:CBS 281.96
Isolated from seed of Glycine max (Leguminosa), cv CMM 60, grown in Thailand, 1990
Classification: Ascomycota, Discomycetes, Rhytismatales, Rhytismataceae

Ascobolus stictoideus Speg.
Isolated from goose dung, Svalbard, Norway
Classification: Ascomycota, Discomycetes, Pezizales, Asc bolaceae

Saccobolus dilutellus (Fuck.) Sacc.
Deposit of strain: Acc No CBS 275.96
Classification: Ascomycota, Discomycetes, Pezizales, Ascobolaceae

Penicillium verruculosum Peyronel
Ex on Acc No of species: ATCC 62396
Classification: Ascomycota, Plectomycetes, Eurotiales, Trichocomaceae

Penicillium chrysogenum Thom
Acc No of Strain: ATCC 9480
Classification: Ascomycota, Plectomycetes, Eurotiales, Trichocomaceae

Thermomyces verrucosus Pugh et al
Deposit of Strain, Acc No.: CBS 285.96
Classification: Ascomycota, Plectomycetes, Eurotiales, (family unclassified; affiliation based on 18S RNA, sequencing and homologies)

Xylaria hypoxylon L. ex Greville
Deposit of Strain, Acc No: CBS 284.96
Classification: Ascomycota, Pyrenomycetes, Xylariales, Xylariaceae

Poronia punctata (Fr.ex L.) Fr.
Classification: Ascomycota, Pyrenomycetes, Xylariales, Xylariaceae

Nodulisporum sp
Isolated from leaf of Camellia reticulatá (Theaceae, Guttiferales), grown in Kunming Botanical Garden, Yunnan Province, China
Classification: Ascomycota, Pyrenomycetes, Xylariales, Xylariaceae

Cylindrocarpon sp
Isolated from marine sample, the Bahamas
Classification: Ascomycota, Pyrenomycetes, Hypocreales (unclassified)

Acremonium sp
Deposit of Strain, Acc. No.: CBS 478.94
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Fusarium anguioides Sherbakoff
Acc No of strain: IFO 4467
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Fusarium poae (Peck) Wr.
Ex on Acc No of species: ATCC 60883
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Fusarium solani (Mart.)Sacc.emnd.Snyd & Hans.
Acc No of strain: IMI 107.511
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Fusarium oxysporum ssp lycopersici (Sacc.)Snyd. & Hans.
Acc No of strain: CBS 645.78
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Fusarium oxysporum ssp passiflora
Acc No of strain: CBS 744.79
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Gliocladium catenulatum Gillman & Abbott
Acc. No. of strain: CBS 227.48

Classification: Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae

Nectria pinea Dingley
Deposit of Strain, Acc. No. CBS 279.96
Classification: Ascomycota, Pyrenomycetes, Hypocreales, Nectriaceae

Volutella colletotrichoides
Acc No of Strain: CBS 400.58
Classification: Ascomycota, Pyrenomycetes, Hypocreales (unclassified)

Sordaria macrospora Auerswald
Ex on Acc No of species: ATCC 60255
Classification: Ascomycota, Pyrenomycetes, Sordariales, Sordariaceae

Sordaria fimicola (Roberge) Cesati et De Notaris
Ex on Acc. No. for the species: ATCC 52644
Isolated from dung by H.Dissing, ISP, KU, Denmark
Classification: Ascomycota, Pyrenomycetes, Sordariales, Sordariaceae

Humicola grisea Traeen
ex on Acc No for the species: ATCC 22726
Source: Hatfield Polytechnic
Classification: Ascomycota, Pyrenomycetes, Sordariales, (fam. unclassified)

Humicola nigrescens Omvik
Acc No of strain: CBS 819.73
Classification: Ascomycota, Pyrenomycetes, Sordariales, (fam. unclassified)

Scytalidium thermophilum (Cooney et Emerson) Austwick
Acc No of strain: ATCC 28085

Classification: Ascomycota, Pyrenomycetes, Sordariales, (fam. unclassified)

Thielavia thermophila Fergus et Sinden
(syn Corynascus thermophilus)
Acc No of strain: CBS 174.70, IMI 145.136
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae
Isolated from Mushroom compost

Thielavia terrestris (Appinis) Malloch et Cain
Acc No of strain: NRRL8126
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Cladorrhinum foecundissimum Saccardo et Marchal
Ex on Acc No of species: ATCC 62373
Classification: Ascomycota, Pyrenomycetes, Sordariales, Lasiosphaeriaceae
Isolated from leaf of Selandin sp. (Compositaceae, Asterales), Dallas Mountain, Jamaica

Syspastospora boninensis
Acc No of strain: NKBC 1515 (Nippon University, profe Tubaki Collection)
Classification: Ascomycota, Pyrenomycetes, Sordariales, Cerastomataceae

Chaetomium cuniculorum Fuckel
Acc. No. of strain: CBS 799.83
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Chaetomium brasiliense Batista et Potual
Acc No of strain: CBS 122.65
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Chaetomium murorum Corda
Acc No of strain: CBS 163.52
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Chaetomium virescens (von Arx) Udagawa
Acc.No. of strain: CBS 547.75
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Myceliophthora thermophila (Apinis) Oorschot
Deposit of Strain, Acc No:CBS 117.65
Classification: Ascomycota, Pyrenomycetes, Sordariales, Chaetomiaceae

Nigrospora sp
Deposit of strain, Acc No: CBS 272.96
Isolated from leaf of Artocarpus altilis, Moraceae, Urticales grown in Christiana, Jamaica
Classification: Ascomycota, Pyrenomycetes, Trichosphaeriales, (family unclassified)

Nigrospora sp
Isolated from leaf of Pinus yuannanensis, Botanical Garden, Kuning, Yunnan.
Classification: Ascomycota, Pyrenomycetes, Trichosphaeriales, Abietaceae, Pinales.

Diaporthe syngenesia
Deposit of strain, Acc No: CBS 278.96
Classification: Ascomycota, Pyrenomycetes, Diaporthales, Valsaceae

Colletotrichum lagenarium (Passerini) Ellis et Halsted
syn Glomerella cingulata var orbiculare Jenkins et Winstead
Ex on acc No of species: ATCC 52609

Classification: Ascomycota, Pyrenomycetes, Phyllachorales

Exidia glandulosa Fr.
Deposit of Strain, Acc No: CBS 277.96
Classification: Basidiomycota, Hymenomycetes, Auriculariales, Exidiaceae

Crinipellis scabella (Alb.&Schw.:Fr.)Murr
Deposit of strain: Acc No CBS 280.96
Classification: Basidiomycota, Hymenomycetes, Agaricales,

Panaeolus retirugis (Fr.) Gill.
Acc.No. of strain: CBS 275.47
Classification: Basidiomycota, Hymenomycetes, Agaricales, Coprinaceae

Fomes fomentarius (L.) Fr.
Deposit of strain: Acc No. CBS 276.96
Classification: Basidiomycota, Hymenomycetes, Aphyllophorales,
Fomitaceae

Spongipellis sp.
Deposit of Strain: Acc No CBS 283.96
Classification: Basidiomycota, Hymenomycetes, Aphyllophorales,
Bjerkanderaceae (identified and affiliated taxonomically by 18S sequence and homology)

Trametes sanguinea (Fr.) Lloyd
syn: Polyporus sanguineus; Pycnoporus sanguineus (L.:Fr.) Murrill
Acc No of strain: AKU 5062 (Kyoto University Culture Collection)
Classification: Basidiomycota, Aphyllophorales, Polyporaceae

Schizophyllum commune Fr
Acc. No. of species: ATCC 38548
Classification: Basidiomycota, Aphyllophorales, Schizophyllaceae

Rhizophlyctis rosea (de Bary & Wor) Fischer
Deposit of Strain: Acc No.: CBS 282.96
Classification: Chytridiomycota, Chytridiomycetes, Spizellomycetales, Spizellomycetaceae

Rhizomucor pusillus (Lindt) Schipper
syn: Mucor pusillus
Acc No of strain: IFO 4578
Ex on Acc No of species: ATCC 46883
Classification: Zygomycota, Zygomycetes, Mucorales, Mucoraceae

Phycomyces nitens (Kunze) van Tieghem & Le Monnier
Acc No of strain: IFO 4814
Ex on Acc No of species: ATCC 16327
Classification: Zygomycota, Zygomycetes, Mucorales, Mucoraceae

Chaetostylum fresenii van Tieghem & Le Monnier
syn. Helicostylum fresenii
Acc No of strain NRRL 2305
Classification: Zygomycota, Zygomycetes, Mucorales, Thamnidiaceae

Unclassified:

Trichothecium roseum
Acc No of strain: IFO 5372

Coniothecium sp
Endophyte, isolated from leaf of unidentifed higher

plant, growing in Kunming, Yunnan, China

Unclassified and Un-identified:

Deposit of strain, Acc No.: CBS 271.96
Isolated from leaf of Artocarpus altilis (Moraceae, Urticales), grown in Christiana, Jamaica

Deposit of strain, Acc No.: CBS 273.96
Isolated from leaf of Pimenta dioica (Myrtaceae, Myrtales) grown in Dallas Mountain, Jamaica

Deposit of strain: CBS 270.96
Isolated from leaf of Pseudocalymma alliaceum (Bignoniaceae, Solanales) growing in Dallas Mountain, Jamaica

Other strains:
Escherichia coli MC1061 and DH10B.

Yeast strain: The *Saccharomyces cerevisiae* strain used was W3124 (MATα; ura 3-52; leu 2-3, 112; his 3-D200; pep 4-1137; prc1::HIS3; prb1:: LEU2; cir+).

Plasmids:
The *Aspergillus* expression vector pHD414 is a derivative of the plasmid p775 (described in EP 238 023). The construction of pHD414 is further described in WO 93/11249.

pYES 2.0 (Invitrogen)

pA2C477, pA2C193, pA2C357, pA2C371, pA2C385, pA2C475, pA2C488, pA2C502 (See example 1, 2, 3 and 4).

Isolation of the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16, or 19 respectively:

The full length DNA sequence, comprising the cDNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, 16 or 19, respectively, coding for the endoglucanase of the invention, can be obtained from the deposited organism *S. cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *E. coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively, by extraction of plasmid DNA by methods known in the art (Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY).

PCR primers for molecular screening of cellulases of the present invention:

The four degenerate, deoxyinosine-containing oligonucleotide primers (sense; s and antisense; as1, as2 and as3) corresponding to four highly conserved amino acid regions found in the deduced amino acid sequences of *Thielavia terrestris* cellulase, *Myceliophthora thermophilum* cellulase, and two cellulases from *Acremonium sp*. The residues are numbered according to the *Myceliophthora thermophilum* sequence. The deoxyinosines are depicted by an I in the primer sequences, and the restriction sites are underlined.

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           27                                                          35
       NH2  -Thr   Arg    Tyr    Trp    Asp    Cys    Cys    Lys     Pro/Thr- COOH

s 5'-CCCCAAGCTT ACI    AGI    TAC    TGG    GAC    TGC    TGC    AAA     AC -3'
      HindIII      C        T              T      T      T      G     C


            106                                111
       NH2    -Trp   Cys    Cys    Ala    Cys    Tyr- COOH

       as1 3'- CC    ACA    ACA    CGI    ACA    AT   AGATCTGATC -5'
                       G      G             G          XbaI


            145                                                            152
      NH2       -Pro    Gly    Gly    Gly    Leu/Val  Gly    Ile/Leu    Phe- COOH

      as2 3'-   GGI     CCI    CCI    CCI    AAI      CCI    AAI        AA   AGATCTGATC -5'
                                             C               G               XbaI
                                             G               T


           193                                      198
      NH2    -Trp    Arg    Phe/Tyr    Asp    Trp    Phe- COOH

      as3 3'- CC     GCI    AAA        CTA    ACC    AAA  AGATCTGATC -5'
                     T        TG         G             G   XbaI
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Molecular screening by polymerase chain reaction (PCR):

In vitro amplification of genomic DNA and double-stranded cDNA.

Directional, double-stranded cDNA was synthesized from 5 μg of poly(A)$^{+}$RNA as described below. Genomic DNA was isolated according to Yelton *et al.*

Approximately 10 to 20 ng of double-stranded, cellulase-induced cDNA or 100 to 200 ng of genomic DNA from a selection of fungal strains was PCR amplified in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % (w/v) gelatin) containing 200 μM of each dNTP and 100 pmol of each degenerate primer in three combinations:

1)
sense,
5'-CCCCAAGCTTACI$^{A}/_{C}$GITA$^{C}/_{T}$TGGGA$^{C}/_{T}$TG$^{C}/_{T}$TG$^{C}/_{T}$AA$^{A}/_{G}$$^{A}/_{C}$C-3'
antisense 1,
5'- CTAGTCTAGATA$^{A}/_{G}$CAIGC$^{A}/_{G}$CA$^{A}/_{G}$CACC -3'; or

2)
sense,
5'- CCCCAAGCTTACI$^{A}/_{C}$GITA$^{C}/_{T}$TGGGA$^{C}/_{T}$TG$^{C}/_{T}$TG$^{C}/_{T}$AA$^{A}/_{G}$$^{A}/_{C}$C-3'
antisense 2,
CTAGTCTAGAAAIA$^{A}/_{G}$/^{T}ICCIA^{A}/^{C}/^{G}ICCICCICCIGG -3'; or

3)
sense,
5'- CCCCAAGCTTACI$^{A}/_{C}$GITA$^{C}/_{T}$TGGGA$^{C}/_{T}$TG$^{C}/_{T}$TG$^{C}/_{T}$AA$^{A}/_{G}$$^{A}/_{C}$C-3'
antisense 3,
5'- CTAGTCTAGAIAACCA$^{A}/_{G}$TCA$^{A}/_{G}$$^{A}/_{T}AIC^{G}/_{T}$CC -3;

a DNA thermal cycler (Landgraf, Germany) and 2.5 units of Taq polymerase (Perkin-Elmer, Cetus, USA). Thirty cycles of PCR were performed using a cycle profile of denaturation at 94 °C for 1 min, annealing at 64 °C for 2

min, and extension at 72 °C for 3 min. Ten-μl aliquots of the amplification products were analyzed by electrophoresis in 3 % agarose gels (NuSieve, FMC) with HaeIII-digested ϕX174 RF DNA as a size marker.

Direct sequencing of the PCR products. Eighty-μl aliquots of the PCR products were purified using the QIAquick PCR purification kit (Qiagen, USA) according to the manufacturer's instructions. The nucleotide sequences of the amplified PCR fragments were determined directly on the purified PCR products by the dideoxy chain-termination method, using 50-150 ng template, the Taq deoxy-terminal cycle sequencing kit (Perkin-Elmer, USA), fluorescent labeled terminators and 5 pmol of the sense primer: 5'- CCCCAAGCTTACI$^{A}/_{C}$GITA$^{C}/_{T}$TGGGA$^{C}/_{T}$T-G$^{C}/_{T}$TG$^{C}/_{T}$AA$^{A}/_{G}{}^{A}/_{C}$C-3'. Analysis of the sequence data were performed according to Devereux *et al*.

Cloning by polymerase chain reaction (PCR): Subcloning of PCR fragments.

Twentyfive-μl aliquots of the PCR products generated as described above were electrophoresed in 0.8 % low gelling temperature agarose (SeaPlaque GTG, FMC) gels, the relevant fragments were excised from the gels, and recovered by agarase treatment by adding 0.1 vol of 10 x agarase buffer (New England Biolabs) and 2 units per 100 μl molten agarose to the sample, followed by incubation at 45 °C for 1.5 h. The sample was phenol and chloroform extracted, and precipitated by addition of 2 vols of 96 % EtOH and 0.1 of 3 M NaAc, pH 5.2. The PCR fragments were recovered by centrifugation, washed in 70 % EtOH, dried and resuspended in 20 μl of restriction enzyme buffer (10 mM Tris-HCl, 10 mM MgCl2, 50 mM NaCl, 1 mM DTT). The fragments were digested with *Hin*dIII and *Xba*I, phenol and chloroform extracted, recovered by precipitation with 2 vols of 96 % EtOH and 0.1 of 3 M

NaAc, pH 5.2, and subcloned into *Hin*dIII/*Xba*I-cleaved pYES 2.0 vector.

Screening of cDNA libraries and characterization of the positive clones. cDNA libraries in S. cerevisiae or E. coli, constructed as described below, were screened by colony hybridization (Sambrook, 1989) using the corresponding random-primed (Feinberg and Vogelstein) ^{32}P-labeled (>1 x 10^9 cpm/μg) PCR products as probes. The hybridizations were carried out in 2 x SSC (Sambrook, 1989), 5 x Denhardt's solution (Sambrook, 1989), 0.5 % (w/v) SDS, 100 μg/ml denatured salmon sperm DNA for 20 h at 65°C followed by washes in 5 x SSC at 25°C (2 x 15 min), 2 x SSC, 0.5 % SDS at 65°C (30 min), 0.2 x SSC, 0.5 % SDS at 65°C (30 min) and finally in 5 x SSC (2 x 15 min) at 25°C. The positive cDNA clones were characterized by sequencing the ends of the cDNA inserts with pYES 2.0 polylinker primers (Invitrogen, USA), and by determining the nucleotide seuence of the longest cDNA from both strands by the dideoxy chain termination method (Sanger et al.) using fluorescent labeled terminators. Qiagen purified plasmid DNA (Qiagen, USA) was sequenced with the Taq deoxy terminal cycle sequencing kit (Perkin Elmer, USA) and either pYES 2.0 polylinker primers (Invitrogen, USA) or synthetic oligonucleotide primers using an Applied Biosystems 373A automated sequencer according to the manufacturers instructions. Analysis of the sequence data was performed according to Devereux *et al*.

Extraction of total RNA was performed with guanidinium thiocyanate followed by ultracentrifugation through a 5.7 M CsCl cushion, and **isolation of poly(A)$^+$RNA** was carried out by oligo(dT)-cellulose affinity chromatography using the procedures described in WO 94/14953.

cDNA synthesis: Double-stranded cDNA was synthesized from 5 μg poly(A)$^+$ RNA by the RNase H method (Gubler and

Hoffman (1983) Gene 25:263-269, Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY) using the hair-pin modification developed by F. S. Hagen (pers. comm.). The poly(A)$^+$ RNA (5 μg in 5 μl of DEPC-treated water) was heated at 70°C for 8 min. in a pre-siliconized, RNase-free Eppendorph tube, quenched on ice and combined in a final volume of 50 μl with reverse transcriptase buffer (50 mM Tris-Cl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, Bethesda Research Laboratories) containing 1 mM of dATP, dGTP and dTTP and 0.5 mM 5-methyl-dCTP (Pharmacia), 40 units human placental ribonuclease inhibitor (RNasin, Promega), 1.45 μg of oligo(dT)$_{18}$-Not I primer (Pharmacia) and 1000 units SuperScript II RNase H reverse transcriptase (Bethesda Research Laboratories). First-strand cDNA was synthesized by incubating the reaction mixture at 45°C for 1 hour. After synthesis, the mRNA:cDNA hybrid mixture was gelfiltrated through a MicroSpin S-400 HR (Pharmacia) spin column according to the manufacturer's instructions.

After the gelfiltration, the hybrids were diluted in 250 μl second strand buffer (20 mM Tris-Cl, pH 7.4, 90 mM KCl, 4.6 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, 0.16 mM βNAD+) containing 200 μM of each dNTP, 60 units *E. coli* DNA polymerase I (Pharmacia), 5.25 units RNase H (Promega) and 15 units *E. coli* DNA ligase (Boehringer Mannheim). Second strand cDNA synthesis was performed by incubating the reaction tube at 16°C for 2 hours and additional 15 min. at 25°C. The reaction was stopped by addition of EDTA to a final concentration of 20 mM followed by phenol and chloroform extractions.

Mung bean nuclease treatment: The double-stranded cDNA was precipitated at -20°C for 12 hours by addition of 2 vols 96% EtOH, 0.2 vol 10 M NH_4Ac, recovered by centrifugation, washed in 70% EtOH, dried and resuspended

in 30 μl Mung bean nuclease buffer (30 mM NaAc, pH 4.6, 300 mM NaCl, 1 mM $ZnSO_4$, 0.35 mM DTT, 2% glycerol) containing 25 units Mung bean nuclease (Pharmacia). The single-stranded hair-pin DNA was clipped by incubating the reaction at 30°C for 30 min., followed by addition of 70 μl 10 mM Tris-Cl, pH 7.5, 1 mM EDTA, phenol extraction and precipitation with 2 vols of 96% EtOH and 0.1 vol 3 M NaAc, pH 5.2 on ice for 30 min.

Blunt-ending with T4 DNA polymerase: The double-stranded cDNAs were recovered by centrifugation and blunt-ended in 30 μl T4 DNA polymerase buffer (20 mM Tris-acetate, pH 7.9, 10 mM MgAc, 50 mM KAc, 1 mM DTT) containing 0.5 mM of each dNTP and 5 units T4 DNA polymerase (New England Biolabs) by incubating the reaction mixture at 16°C for 1 hour. The reaction was stopped by addition of EDTA to a final concentration of 20 mM, followed by phenol and chloroform extractions, and precipitation for 12 hours at -20°C by adding 2 vols 96% EtOH and 0.1 vol 3 M NaAc pH 5.2.

Adaptor ligation, Not I digestion and size selection: After the fill-in reaction the cDNAs were recovered by centrifugation, washed in 70% EtOH and dried. The cDNA pellet was resuspended in 25 μl ligation buffer (30 mM Tris-Cl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP) containing 2.5 μg non-palindromic BstXI adaptors (Invitrogen) and 30 units T4 ligase (Promega) and incubated at 16°C for 12 hours. The reaction was stopped by heating at 65°C for 20 min. and then cooling on ice for 5 min. The adapted cDNA was digested with Not I restriction enzyme by addition of 20 μl water, 5 μl 10x Not I restriction enzyme buffer (New England Biolabs) and 50 units Not I (New England Biolabs), followed by incubation for 2.5 hours at 37°C. The reaction was stopped by heating at 65°C for 10 min. The cDNAs were size-fractionated by gel electrophoresis on a 0.8%

SeaPlaque GTG low melting temperature agarose gel (FMC) in 1x TBE to separate unligated adaptors and small cDNAs. The cDNA was size-selected with a cut-off at 0.7 kb and rescued from the gel by use of β-Agarase (New England Biolabs) according to the manufacturer's instructions and precipitated for 12 hours at -20°C by adding 2 vols 96% EtOH and 0.1 vol 3 M NaAc pH 5.2.

Construction of libraries: The directional, size-selected cDNA was recovered by centrifugation, washed in 70% EtOH, dried and resuspended in 30 µl 10 mM Tris-Cl, pH 7.5, 1 mM EDTA. The cDNAs were desalted by gelfiltration through a MicroSpin S-300 HR (Pharmacia) spin column according to the manufacturer's instructions. Three test ligations were carried out in 10 µl ligation buffer (30 mM Tris-Cl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP) containing 5 µl double-stranded cDNA (reaction tubes #1 and #2), 15 units T4 ligase (Promega) and 30 ng (tube #1), 40 ng (tube #2) and 40 ng (tube #3, the vector background control) of BstXI-NotI cleaved pYES 2.0 vector. The ligation reactions were performed by incubation at 16°C for 12 hours, heating at 70°C for 20 min. and addition of 10 µl water to each tube. 1 µl of each ligation mixture was electroporated into 40 µl electrocompetent *E. coli* DH10B cells (Bethesda research Laboratories) as described (Sambrook et al. (1989) Molecular cloning: A laboratory manual, Cold Spring Harbor lab., Cold Spring Harbor, NY). Using the optimal conditions a library was established in *E. coli* consisting of pools. Each pool was made by spreading transformed *E. coli* on LB+ampicillin agar plates giving 15.000-30.000 colonies/plate after incubation at 37°C for 24 hours. 20 ml LB+ampicillin was added to the plate and the cells were suspended herein. The cell suspension was shaked in a 50 ml tube for 1 hour at 37°C. Plasmid DNA was isolated from the cells according to the manufacturer's instructions using QIAGEN plasmid kit and stored at -20°C.

1 μl aliquots of purified plasmid DNA (100 ng/μl) from individual pools were transformed into *S. cerevisiae* W3124 by electroporation (Becker and Guarante (1991) Methods Enzymol. 194:182-187) and the transformants were plated on SC agar containing 2% glucose and incubated at 30°C.

Identification of positive colonies: After 3-5 days of growth, the agar plates were replica plated onto a set of SC + galactose-uracil agar plates containing 0.1% AZCL HE cellulose. These plates were incubated for 3-7 days at 30°C. Endoglucanase positive colonies were identified as colonies surrounded by a blue halo.

Cells from enzyme-positive colonies were spread for single colony isolation on agar, and an enzyme-producing single colony was selected for each of the endoglucanase-producing colonies identified.

Characterization of positive clones: The positive clones were obtained as single colonies, the cDNA inserts were amplified directly from the yeast colony using biotinylated polylinker primers, purified by magnetic beads (Dynabead M-280, Dynal) system and characterized individually by sequencing the 5'-end of each cDNA clone using the chain-termination method (Sanger et al. (1977) Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467) and the Sequenase system (United States Biochemical).

The nucleotide sequence was determined of the longest cDNA from both strands by the dideoxy chain termination method (Sanger et al.) using fluorescent labeled terminators. Plasmid DNA was rescued by transformation into *E. coli* as described below. Qiagen purified plasmid DNA (Qiagen, USA) was sequenced with the Taq deoxy terminal cycle sequencing kit (Perkin Elmer, USA) and either pYES 2.0 polylinker primers (Invitrogen, USA) or

synthetic oligonucleotide primers using an Applied Biosystems 373A automated sequencer according to the manufacturers instructions. Analysis of the sequence data was performed according to Devereux *et al*.

Isolation of a cDNA gene for expression in Aspergillus: An endoglucanase-producing yeast colony was inoculated into 20 ml YPD broth in a 50 ml glass test tube. The tube was shaken for 2 days at 30°C. The cells were harvested by centrifugation for 10 min. at 3000 rpm.

DNA was isolated according to WO 94/14953 and dissolved in 50 µl water. The DNA was transformed into *E. coli* by standard procedures. Plasmid DNA was isolated from *E. coli* using standard procedures, and analyzed by restriction enzyme analysis. The cDNA insert was excised using appropriate restriction enzymes and ligated into an Aspergillus expression vector.

Transformation of *Aspergillus oryzae* or *Aspergillus niger*

Protoplasts may be prepared as described in WO 95/02043, p. 16, line 21 - page 17, line 12, which is hereby incorporated by reference.

100 µl of protoplast suspension is mixed with 5-25 µg of the appropriate DNA in 10 µl of STC (1.2 M sorbitol, 10 mM Tris-HCl, pH = 7.5, 10 mM $CaCl_2$). Protoplasts are mixed with p3SR2 (an *A. nidulans* amdS gene carrying plasmid). The mixture is left at room temperature for 25 minutes. 0.2 ml of 60% PEG 4000 (BDH 29576), 10 mM $CaCl_2$ and 10 mM Tris-HCl, pH 7.5 is added and carefully mixed (twice) and finally 0.85 ml of the same solution is added and carefully mixed. The mixture is left at room temperature for 25 minutes, spun at 2500 g for 15 minutes and the pellet is resuspended in 2 ml of 1.2 M sorbitol. After one more sedimentation the protoplasts are spread

on minimal plates (Cove, Biochem. Biophys. Acta 113 (1966) 51-56) containing 1.0 M sucrose, pH 7.0, 10 mM acetamide as nitrogen source and 20 mM CsCl to inhibit background growth. After incubation for 4-7 days at 37°C spores are picked and spread for single colonies. This procedure is repeated and spores of a single colony after the second reisolation is stored as a defined transformant.

Test of *A. oryzae* transformants

Each of the transformants were inoculated in 10 ml YPM and propagated. After 2-5 days of incubation at 37°C, 10 ml supernatant was removed. The endoglucanase activity was identified by AZCL HE cellulose as described above.

Hybridization conditions (to be used in evaluating property ii) of the DNA construct of the invention): Suitable conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5 x SSC (standard saline citrate) for 10 min, and prehybridization of the filter in a solution of 5 x SSC (Sambrook et al. 1989), 5 x Denhardt's solution (Sambrook et al. 1989), 0.5 % SDS and 100 μg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) *Anal. Biochem.* **132**:6-13), ^{32}P-dCTP-labeled (specific activity > 1 x 10^9 cpm/μg) probe for 12 hours at ca. 45°C. The filter is then washed two times for 30 minutes in 2 x SSC, 0.5 % SDS at preferably not higher than 50°C, more preferably not higher than 55°C, more preferably not higher than 60°C, more preferably not higher than 65°C, even more preferably not higher than 70°C, especially not higher than 75°C.
The nucleotide probe to be used in the hybridization is the DNA sequence corresponding to the endoglucanase

encoding part of the DNA sequence shown in SEQ ID No. 1, 4, 6, 8, 10, 12, or 16, resepctively, and/or the DNA sequence obtainable from the plasmid in *S. cerevisiae*, DSM 9770, DSM 10082, DSM 10080, DSM 10081, *E. coli*, DSM 10512, DSM 10511, DSM 10571 or DSM 10576, respectively.

Immunological cross-reactivity: Antibodies to be used in determining immunological cross-reactivity may be prepared by use of a purified cellulase. More specifically, antiserum against the cellulase of the invention may be raised by immunizing rabbits (or other rodents) according to the procedure described by N. Axelsen et al. in: A Manual of Quantitative Immunoelectrophoresis, Blackwell Scientific Publications, 1973, Chapter 23, or A. Johnstone and R. Thorpe, Immunochemistry in Practice, Blackwell Scientific Publications, 1982 (more specifically pp. 27-31). Purified immunoglobulins may be obtained from the antisera, for example by salt precipitation ($(NH_4)_2SO_4$), followed by dialysis and ion exchange chromatography, e.g. on DEAE-Sephadex. Immunochemical characterization of proteins may be done either by Outcherlony double-diffusion analysis (O. Ouchterlony in: Handbook of Experimental Immunology (D.M. Weir, Ed.), Blackwell Scientific Publications, 1967, pp. 655-706), by crossed immunoelectrophoresis (N. Axelsen et al., supra, Chapters 3 and 4), or by rocket immunoelectrophoresis (N. Axelsen et al., Chapter 2).

Media

YPD: 10 g yeast extract, 20 g peptone, H_2O to 900 ml. Autoclaved, 100 ml 20% glucose (sterile filtered) added.

YPM: 10 g yeast extract, 20 g peptone, H_2O to 900 ml. Autoclaved, 100 ml 20% maltodextrin (sterile filtered) added.

10 x Basal salt: 75 g yeast nitrogen base, 113 g succinic acid, 68 g NaOH, H_2O ad 1000 ml, sterile filtered.

SC-URA: 100 ml 10 x Basal salt, 28 ml 20% casamino acids without vitamins, 10 ml 1% tryptophan, H_2O ad 900 ml, autoclaved, 3.6 ml 5% threonine and 100 ml 20% glucose or 20% galactose added.

SC-URA agar: SC-URA, 20 g/l agar added.

PD agar: 39g potato dextrose agar, DIFCO 0013; add deionized water up to 1000ml; autoclave (121°C for 15-20 min).

PC agar: Potatoes and carrots (grinded, 20 g of each) and water, added up to 1000ml, are boiled for 1 hr; agar (20g/l of Merck 1614); autoclave (121°C for 20 min)

PC liquid broth: as PC agar but without the Agar

PD liquid broth: 24g potato dextrose broth, Difco 0549, deionized water up to 1000ml; autoclave (121°C for 15-20 min)

PC and PD liquid broth with cellulose: add 30 g Solcafloc (Dicacel available from Dicalite-Europe-Nord, 9000 Gent, Belgium) per 1000ml

PB-9 liquid broth: 12 g Rofec (Roquette 101-0441) and 24 g glucose are added to 1000ml water; pH is adjusted to 5.5; 5ml mineral oil and 5 g $CaCo_3$ are added per 1000ml. autoclave (121°C for 40 min)

YPG liquid broth: 4g yeast extract (Difco 0127), 1g KH_2PO_4 (Merck4873), 0.5g $MgSO_4$.7H2O Merck 5886, 15g Dextrose, Roquette 101-0441, 0.1ml Pluronic (101-3088); deionized water up to 1000ml; autoclave (20min at 121°C)

Dilute salt solution (DS): Make up two stock solutions: P-stock: 13.61g KH_2PO_4; 13.21g $(NH_4)2PO_4$, 17.42g KH_2PO_4; deionized water up to 100ml
Ca/Mg stock: 7.35g $CaCl_2$, $2H_2O$, 10.17g $MgCl_2$, $6H_2O$, deionized water up to 100ml; pH adjusted to 7.0; autoclaving (121°C; 20min)
Mix 0..5ml P-stock with 0.1ml Ca/Mg stock add deionized water up to 1000ml

AZCL HE cellulose (Megazyme, Australia).

Uses

During washing and wearing, dyestuff from dyed fabrics or garment will conventionally bleed from the fabric which then looks faded and worn. Removal of surface fibers from the fabric will partly restore the original colours and looks of the fabric. By the term "colour clarification", as used herein, is meant the partly restoration of the initial colours of fabric or garment throughout multiple washing cycles.

The term "de-pilling" denotes removing of pills from the fabric surface.

The term "soaking liquor" denotes an aqueous liquor in which laundry may be immersed prior to being subjected to a conventional washing process. The soaking liquor may contain one or more ingredients conventionally used in a washing or laundering process.

The term "washing liquor" denotes an aqueous liquor in which laundry is subjected to a washing process, i.e. usually a combined chemical and mechanical action either manually or in a washing machine. Conventionally, the

washing liquor is an aqueous solution of a powder or liquid detergent composition.

The term "rinsing liquor" denotes an aqueous liquor in which laundry is immersed and treated, conventionally immediately after being subjected to a washing process, in order to rinse the laundry, i.e. essentially remove the detergent solution from the laundry. The rinsing liquor may contain a fabric conditioning or softening composition.

The laundry subjected to the method of the present invention may be conventional washable laundry. Preferably, the major part of the laundry is sewn or unsewn fabrics, including knits, wovens, denims, yarns, and toweling, made from cotton, cotton blends or natural or manmade cellulosics (e.g. originating from xylan-containing cellulose fibers such as from wood pulp) or blends thereof. Examples of blends are blends of cotton or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, flax/linen, jute, cellulose acetate fibers, lyocell).

Detergent Compositions

According to one aspect of the present invention, the present endoglucanases may typically be components of a detergent composition. As such, they may be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or protected enzymes. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo

Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or nonaqueous.

The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzenesulfonate (LAS), alpha-olefinsulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkanesulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamine oxide,

ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes such as amylase, lipase, cutinase, protease, peroxidase, and oxidase, e.g. laccase.

The detergent may contain 1-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

The detergent may comprise one or more polymers. Examples are carboxymethylcellulose (CMC), poly(vinylpyrrolidone) (PVP), polyethyleneglycol (PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

The detergent may contain a bleaching system which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzenesulfonate (NOBS). Alternatively, the bleaching system may comprise peroxyacids of, e.g., the amide, imide, or sulfone type.

The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a

boric acid derivative such as, e.g., an aromatic borate ester, and the composition may be formulated as described in, e.g., WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil-redeposition agents, dyes, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11.

Particular forms of detergent compositions within the scope of the invention include:

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	7 - 12%
Alcohol ethoxysulfate (e.g. C_{12-18} alcohol, 1-2 EO) or alkyl sulfate (e.g. C_{16-18})	1 - 4%
Alcohol ethoxylate (e.g. C_{14-15} alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na_2CO_3)	14 - 20%
Soluble silicate (as $Na_2O,2SiO_2$)	2 - 6%
Zeolite (as $NaAlSiO_4$)	15 - 22%
Sodium sulfate (as Na_2SO_4)	0 - 6%
Sodium citrate/citric acid (as $C_6H_5Na_3O_7/C_6H_8O_7$)	0 - 15%
Sodium perborate (as $NaBO_3.H_2O$)	11 - 18%
TAED	2 - 6%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener, photobleach)	0 - 5%

2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	6 - 11%
Alcohol ethoxysulfate (e.g. C_{12-18} alcohol, 1-2 EO or alkyl sulfate (e.g. C_{16-18})	1 - 3%
Alcohol ethoxylate (e.g. C_{14-15} alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na_2CO_3)	15 - 21%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	24 - 34%
Sodium sulfate (as Na_2SO_4)	4 - 10%
Sodium citrate/citric acid (as $C_6H_5Na_3O_7/C_6H_8O_7$)	0 - 15%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 6%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	5 - 9%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO)	7 - 14%
Soap as fatty acid (e.g. C_{16-22} fatty acid)	1 - 3%
Sodium carbonate (as Na_2CO_3)	10 - 17%
Soluble silicate (as $Na_2O,2SiO_2$)	3 - 9%
Zeolite (as $NaAlSiO_4$)	23 - 33%
Sodium sulfate (as Na_2SO4)	0 - 4%
Sodium perborate (as $NaBO_3.H_2O$)	8 - 16%
TAED	2 - 8%
Phosphonate (e.g. EDTMPA)	0 - 1%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener)	0 - 5%

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzenesulfonate (calculated as acid)	8 - 12%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO)	10 - 25%
Sodium carbonate (as Na_2CO_3)	14 - 22%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 5%
Zeolite (as $NaAlSiO_4$)	25 - 35%
Sodium sulfate (as Na_2SO_4)	0 - 10%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

5) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15 - 21%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO or C_{12-15} alcohol, 5 EO)	12 - 18%
Soap as fatty acid (e.g. oleic acid)	3 - 13%
Alkenylsuccinic acid (C_{12-14})	0 - 13%
Aminoethanol	8 - 18%
Citric acid	2 - 8%
Phosphonate	0 - 3%
Polymers (e.g. PVP, PEG)	0 - 3%
Borate (as B_4O_7)	0 - 2%
Ethanol	0 - 3%
Propylene glycol	8 - 14%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. dispersants, suds suppressors, perfume, optical brightener)	0 - 5%

6) An aqueous structured liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15 - 21%
Alcohol ethoxylate (e.g. C_{12-15} alcohol,7 EO, or C_{12-15}alcohol,5 EO)	3 - 9%
Soap as fatty acid (e.g. oleic acid)	3 - 10%
Zeolite (as $NaAlSiO_4$)	14 - 22%
Potassium citrate	9 - 18%
Borate (as B_4O_7)	0 - 2%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. PEG, PVP)	0 - 3%
Anchoring polymers such as, e.g., lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1; MW 3800	0 - 3%
Glycerol	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. dispersants, suds suppressors, perfume, optical brighteners)	0 - 5%

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Fatty alcohol sulfate	5 - 10%
Ethoxylated fatty acid monoethanolamide	3 - 9%
Soap as fatty acid	0 - 3%
Sodium carbonate (as Na_2CO_3)	5 - 10%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	20 - 40%
Sodium sulfate (as Na_2SO_4)	2 - 8%
Sodium perborate (as $NaBO_3.H_2O$)	12 - 18%
TAED	2 - 7%
Polymers (e.g. maleic/acrylic acid copolymer, PEG)	1 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, suds suppressors, perfume)	0 - 5%

8) A detergent composition formulated as a granulate comprising

Linear alkylbenzenesulfonate (calculated as acid)	8 - 14%
Ethoxylated fatty acid monoethanolamide	5 - 11%
Soap as fatty acid	0 - 3%
Sodium carbonate (as Na_2CO_3)	4 - 10%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	30 - 50%
Sodium sulfate (as Na_2SO_4)	3 - 11%
Sodium citrate (as $C_6H_5Na_3O_7$)	5 - 12%
Polymers (e.g. PVP, maleic/acrylic acid copolymer, PEG)	1 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

9) A detergent composition formulated as a granulate comprising

Linear alkylbenzenesulfonate (calculated as acid)	6 - 12%
Nonionic surfactant	1 - 4%
Soap as fatty acid	2 - 6%
Sodium carbonate (as Na_2CO_3)	14 - 22%
Zeolite (as $NaAlSiO_4$)	18 - 32%
Sodium sulfate (as Na_2SO_4)	5 - 20%
Sodium citrate (as $C_6H_5Na_3O_7$)	3 - 8%
Sodium perborate (as $NaBO_3.H_2O$)	4 - 9%
Bleach activator (e.g. NOBS or TAED)	1 - 5%
Carboxymethylcellulose	0 - 2%
Polymers (e.g. polycarboxylate or PEG)	1 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, perfume)	0 - 5%

10) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	15 - 23%
Alcohol ethoxysulfate (e.g. C_{12-15} alcohol, 2-3 EO)	8 - 15%
Alcohol ethoxylate (e.g. C_{12-15} alcohol,7 EO, or C_{12-15}alcohol, 5 EO)	3 - 9%
Soap as fatty acid (e.g. lauric acid)	0 - 3%
Aminoethanol	1 - 5%
Sodium citrate	5 - 10%
Hydrotrope (e.g. sodium toluensulfonate)	2 - 6%
Borate (as B_4O_7)	0 - 2%
Carboxymethylcellulose	0 - 1%
Ethanol	1 - 3%
Propylene glycol	2 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. polymers, dispersants, perfume, optical brighteners)	0 - 5%

11) An aqueous liquid detergent composition comprising

Linear alkylbenzenesulfonate (calculated as acid)	20 - 32%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO, or C_{12-15} alcohol, 5 EO)	6 - 12%
Aminoethanol	2 - 6%
Citric acid	8 - 14%
Borate (as B_4O_7)	1 - 3%
Polymer (e.g. maleic/acrylic acid copolymer, anchoring polymer such as, e.g., lauryl methacrylate-/acrylic acid copolymer)	0 - 3%
Glycerol	3 - 8%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. hydrotropes, dispersants, perfume, optical brighteners)	0 - 5%

12) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Anionic surfactant (linear alkylbenzenesulfonate, alkyl sulfate, alpha-olefinsulfonate, alpha-sulfo fatty acid methyl esters, alkanesulfonates, soap)	25 - 40%
Nonionic surfactant (e.g. alcohol ethoxylate)	1 - 10%
Sodium carbonate (as Na_2CO_3)	8 - 25%
Soluble silicates (as Na_2O, $2SiO_2$)	5 - 15%
Sodium sulfate (as Na_2SO_4)	0 - 5%
Zeolite (as $NaAlSiO_4$)	15 - 28%
Sodium perborate (as $NaBO_3.4H_2O$)	0 - 20%
Bleach activator (TAED or NOBS)	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. perfume, optical brighteners)	0 - 3%

13) Detergent formulations as described in 1) - 12) wherein all or part of the linear alkylbenzenesulfonate is replaced by (C_{12}-C_{18}) alkyl sulfate.

14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

(C_{12}-C_{18}) alkyl sulfate	9 - 15%
Alcohol ethoxylate	3 - 6%
Polyhydroxy alkyl fatty acid amide	1 - 5%
Zeolite (as $NaAlSiO_4$)	10 - 20%
Layered disilicate (e.g. SK56 from Hoechst)	10 - 20%
Sodium carbonate (as Na_2CO_3)	3 - 12%
Soluble silicate (as $Na_2O,2SiO_2$)	0 - 6%
Sodium citrate	4 - 8%
Sodium percarbonate	13 - 22%
TAED	3 - 8%
Polymers (e.g. polycarboxylates and PVP=	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, photo bleach, perfume, suds suppressors)	0 - 5%

15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

$(C_{12}-C_{18})$ alkyl sulfate	4 - 8%
Alcohol ethoxylate	11 - 15%
Soap	1 - 4%
Zeolite MAP or zeolite A	35 - 45%
Sodium carbonate (as Na_2CO_3)	2 - 8%
Soluble silicate (as $Na_2O,2SiO_2$)	0 - 4%
Sodium percarbonate	13 - 22%
TAED	1 - 8%
Carboxymethyl cellulose	0 - 3%
Polymers (e.g. polycarboxylates and PVP)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, phosphonate, perfume)	0 - 3%

16) Detergent formulations as described in 1) - 15) which contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for already specified bleach systems.

17) Detergent compositions as described in 1), 3), 7), 9) and 12) wherein perborate is replaced by percarbonate.

18) Detergent compositions as described in 1), 3), 7), 9), 12), 14) and 15) which additionally contain a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature 369, 1994, pp. 637-639.

19) Detergent composition formulated as a nonaqueous

detergent liquid comprising a liquid nonionic surfactant such as, e.g., linear alkoxylated primary alcohol, a builder system (e.g. phosphate), enzyme and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

The endoglucanase may be incorporated in concentrations conventionally employed in detergents. It is at present contemplated that, in the laundry composition of the invention, the cellulase may be added in an amount corresponding to 0.0001-10 mg (calculated as pure enzyme protein) of cellulase per liter of wash liquor.

According to yet another aspect of the present invention, endoglucanase may typically be a component of a fabric conditioning or softener composition. Examples of conventional softener compositions are disclosed in e.g. EP 0 233 910.

Textile applications

In another embodiment, the present invention relates to use of the endoglucanase of the invention in the bio-polishing process. Bio-Polishing is a specific treatment of the yarn surface which improves fabric quality with respect to handle and appearance without loss of fabric wettability. The most important effects of Bio-Polishing can be characterized by less fuzz and pilling, increased gloss/luster, improved fabric handle, increased durable softness and altered water absorbency. Bio-Polishing usually takes place in the wet processing of the manufacture of knitted and woven fabrics. Wet processing comprises such steps as e.g. desizing, scouring, bleaching, washing, dying/printing and finishing. During each of these steps, the fabric is more or less subjected to mechanical action. In general, after the textiles have

been knitted or woven, the fabric proceeds to a desizing stage, followed by a scouring stage, etc. Desizing is the act of removing size from textiles. Prior to weaving on mechanical looms, warp yarns are often coated with size starch or starch derivatives in order to increase their tensile strength. After weaving, the size coating must be removed before further processing the fabric in order to ensure a homogeneous and wash-proof result. It is known that in order to achieve the effects of Bio-Polishing, a combination of cellulytic and mechanical action is required. It is also known that "super-softness" is achievable when the treatment with a cellulase is combined with a conventional treatment with softening agents. It is contemplated that use of the endoglucanase of the invention for bio-polishing of cellulosic fabrics is advantageous, e.g. a more thorough polishing can be achieved. Bio-polishing may be obtained by applying the method described e.g. in WO 93/20278.

Stone-washing

It is known to provide a "stone-washed" look (localized abrasion of the colour) in dyed fabric, especially in denim fabric or jeans, either by washing the denim or jeans made from such fabric in the presence of pumice stones to provide the desired localized lightening of the colour of the fabric or by treating the fabric enzymatically, in particular with cellulytic enzymes. The treatment with an endoglucanase of the present invention may be carried out either alone such as disclosed in US 4,832,864, together with a smaller amount of pumice than required in the traditional process, or together with perlite such as disclosed in WO 95/09225.

<u>Pulp and paper applications</u>

In the papermaking pulp industry, the endoglucanase of the present invention may be applied advantageously e.g. as follows:

- For debarking: pretreatment with the endoglucanase may degrade the cambium layer prior to debarking in mechanical drums resulting in advantageous energy savings.

- For defibration: treatment of a material containing cellulosic fibers with the endoglucanase prior to refining or beating may result in reduction of the energy consumption due to the hydrolysing effect of the cellulase on the interfibre surfaces. Use of the endoglucanase may result in improved energy savings as compared to the use of known enzymes, since it is believed that the enzyme composition of the invention may possess a higher ability to penetrate fibre walls.

- For fibre modification, i.e. improvement of fibre properties where partial hydrolysis across the fibre wall is needed which requires deeper penetrating enzymes (e.g. in order to make coarse fibers more flexible). Deep treatment of fibers has so far not been possible for high yield pulps e.g. mechanical pulps or mixtures of recycled pulps. This has been ascribed to the nature of the fibre wall structure that prevents the passage of enzyme molecules due to physical restriction of the pore matrix of the fibre wall. It is contemplated that the present endoglucanase is capable of penetrating into the fibre wall.

- For drainage improvement. The drainability of papermaking pulps may be improved by treatment of the pulp with hydrolysing enzymes, e.g. cellulases. Use of

The treatment of lignocellulosic pulp may, e.g., be performed as described in WO 91/14819, WO 91/14822, WO 92/17573 and WO 92/18688.

Degradation of plant material

In yet another embodiment, the present invention relates to use of the endoglucanase and/or enzyme preparation according to the invention for degradation of plant material e.g. cell walls.

It is contemplated that the novel endoglucanase and/or enzyme preparation of the invention is useful in the preparation of wine, fruit or vegetable juice in order to increase yield. Endoglucanases according to the invention may also be applied for enzymatic hydrolysis of various plant cell-wall derived materials or waste materials, e.g. agricultural residues such as wheat-straw, corn cobs, whole corn plants, nut shells, grass, vegetable hulls, bean hulls, spent grains, sugar beet pulp, and the like. The plant material may be degraded in order to improve different kinds of processing, facilitate purification or extraction of other components like purification of beta-glucan or beta-glucan oligomers from cereals, improve the feed value, decrease the water binding capacity, improve the degradability in waste water plants, improve the conversion of e.g. grass and corn to ensilage, etc.

The following examples illustrate the invention.

EXAMPLE 1

Cellulytic enzymes from 4 fungi, belonging to 3 families under two orders within the Ascomycetes were detected by expression cloning; corresponding DNA sequences were determined; the enzymes heterologously expressed, and produced by liquid fermentation, characterized and demonstrated to give good performance in colour clarification assays.

Isolate CBS 117.65, CBS 478.94, NRRL 8126, and ATCC 10523 were grown in shake flask cultures on cellulose enriched potato dextrose broth, incubated for 5 days at 26^{0}C (shaking conditions, 150 rpm).

A. Cloning and expression of an endoglucanase from *Myceliophthora thermophila, Acremonium sp.,* and *Thielavia terrestris* and *Volutella colletotrichoides*

mRNA was isolated from *Myceliophthora thermophila, Acremonium sp., Thielavia terrestris* and *Volutella colletotrichoides*, respectively, grown in a cellulose-containing fermentation medium with agitation to ensure sufficient aeration. Mycelia were harvested after 3-5 days' growth, immediately frozen in liquid nitrogen and stored at -80°C. Libraries from *Myceliophthora thermophila, Acremonium sp., Thielavia terrestris* and *Volutella colletotrichoides*, respectively, each consisting of approx. 10^6 individual clones were constructed in *E. coli* as described with a vector background of 1%.

Plasmid DNA from some of the pools from each library was transformed into yeast, and 50-100 plates containing 250-400 yeast colonies were obtained from each pool.

Endoglucanase-positive colonies were identified and

isolated on SC-agar plates with the AZCL HE cellulose assay. cDNA inserts were amplified directly from the yeast colonies and characterized as described in the Materials and Methods section above.

The DNA sequence of the cDNA encoding the endoglucanase from *Myceliophthora thermophila* is shown in SEQ ID No. 1 and the corresponding amino acid sequence is also shown in SEQ ID No. 1. The cDNA is obtainable from the plasmid in DSM 9770.

The DNA sequence of the cDNA encoding the endoglucanase from *Acremonium sp.* is shown in SEQ ID No. 4 and the corresponding amino acid sequence is shown in SEQ ID No. 5. The cDNA is obtainable from the plasmid in DSM 10082.

The DNA sequence of the cDNA encoding the endoglucanase from *Thielavia terrestris* is shown in SEQ ID No. 8 and the corresponding amino acid sequence is shown in SEQ ID No. 9. The cDNA is obtainable from the plasmid in DSM 10081.

The DNA sequence of the cDNA encoding the endoglucanase from *Volutella colletotrichoides* is shown in SEQ ID No. 16 and the corresponding amino acid sequence is shown in SEQ ID No. 17. The cDNA is obtainable from the plasmid in DSM 10571.

Total DNA was isolated from a yeast colony and plasmid DNA was rescued by transformation of *E. coli* as described above. In order to express the endoglucanases in *Aspergillus*, the DNA was digested with appropriate restriction enzymes, size fractionated on gel, and a fragment corresponding to the endoglucanase gene from *Myceliophthora thermophila, Acremonium sp., Thielavia terrestris* and *Volutella colletotrichoides*, respectively, was purified. The genes were subsequently ligated to

pHD414, digested with appropriate restriction enzymes, resulting in the plasmids pA2C193, pA2C357, pA2C385 and pA2C488, respectively.

After amplification of the DNA in *E. coli* the plasmids were transformed into *Aspergillus oryzae* as described above.

Test of *A. oryzae* transformants
Each of the transformants were tested for endoglucanase activity as described above. Some of the transformants had endoglucanase activity which was significantly larger than the *Aspergillus oryzae* background. This demonstrates efficient expression of the endoglucanases in *Aspergillus oryzae*. The transformants with the highest endoglucanase activity were selected and inoculated in a 500 ml shake flask with YPM media. After 3-5 days of fermentation with sufficient agitation to ensure good aeration, the culture broth was centrifuged for 10 minutes at 2000 g and the supernatant recovered.

B. Determination of endoglucanase activity
The cellulytic activity of the endoglucanase may be determined relative to an analytical standard and expressed in the unit S-CEVU.

Cellulytic enzymes hydrolyse CMC, thereby decreasing the viscosity of the incubation mixture. The resulting reduction in viscosity may be determined by a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France).

Determination of the cellulytic activity, measured in terms of S-CEVU, may be determined according to the analysis method AF 301.1 which is available from the Applicant upon request.

The S-CEVU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C, pH 7.5 using a relative enzyme standard for reducing the viscosity of the CMC substrate.

Assay for determination of endoglucanase activity in terms of SAVI units using phosphoric-acid swollen cellulose (PASC):

Definition:
1 SAVI-U is the amount of enzyme which forms an amount of reducing carbohydrates equivalent to 1 μmol of glucose per minute.

Assay condition:
Enzyme solution: 0,5 ml
4 g/l PASC in 0,1 M Buffer: 2.0 ml
20 min, 40 °C

Sensitivity:
Max 0.1 SAVIU/ml = approx. 1 S-CEVU/ml (CMC viscosity)
Min 0.01 SAVIU/ml = approx. 0.1 S-CEVU/ml

Determination of formation of reducing sugars:

The reducing groups assay was performed according to Lever, M. A new reaction for colormetric determination of carbohydrates. Anal. Biochem. 1972. Vol 47 (273-279). Reagent mixture was prepared by mixing 1,5 gram p-hydroxybenzoic-acide hydracide (PHBAH) with 5 gram sodium tartrate in 100 ml 2 % sodium hydroxide.

Substrate:

PASC stock solution was prepared the following way using

ice cold acetone and phosphoric acid. 5 gram of cellulose (Avicel®) was moistered with water, and 150 ml ice cold 85% ortho-phosphoric acid was added. The mixture was placed in ice bath under slow stirring for 1 hr. Then 100 ml ice cold acetone was added with stirring. The slurry was transferred to a Buchner filter with pyrex sintered disc number 3 and then washed three times with 100 ml ice cold acetone, and sucked as dry as possible after each wash. Finally, the filter cake was washed twice with 500 ml water, sucked as dry as possible after each wash. The PASC was mixed with deionized water to a total volume of 300 ml, blended to homogeneity (using the Ultra Turrax Homogenizer) and stored in refrigerator (up to one month).

Substrate equilibration with buffer: 20 gram phosphoric acid swollen cellulose PASC stock solution was centrifuged for 20 min at 5000 rpm., the supernatant was poured of; the sediment was resuspended in 30 ml of buffer and centrifuged for 20 min. at 5000 rpm., the supernatant was poured of, and the sediment was resuspended in buffer to a total of 60 g corresponding to a substrate concentration of 5 g cellulose/litre.

Buffer for pH 8,5 determination: 0.1 M Barbital.
Buffer for pH 10 determination: 0.1 M Glycine.

Procedure:
1. Dilution of enzyme samples
The enzyme solution is diluted in the same buffer as the substrate.

2. Enzyme reaction
The substrate in buffer solution is preheated for 5 min. at 40°C (2 ml).
Then the enzyme solution (diluted to between 0.2 and 1 S-CEVU/ml) 0,5 ml is added and mixed for 5 sec. Enzymes

blanks are obtained by adding the stop reagent before enzyme solution. Incubate for 20 min. at 40 °C. The reaction is stopped by adding 0.5 ml 2% NaOH solution and mixing for 5 sec.

The samples are centrifuged for 20 min. at 5000 rpm. 1 ml supernatant is mixed with 0.5 ml PHBAH reagent and boiled for 10 min. The test tubes are cooled in a ice water bath.

3. Determination of reducing end groups:

The absorbancy at 410 nm is measured using a spectrophotometer. Blanks are prepared by adding sodium hydroxide before adding enzyme solution.
A standard glucose curve was obtained by using glucose concentrations of 5, 10, 15 and 25 mg/l in the same buffer and adding PHBAH reagent before boiling. The release of reducing glucose equivalent is calculated using this standard curve.

4. Calculation of catalytic activity:

Measure absorbance at 410 nm

1) Standard curve

(Glucose) - (H_2O) vs concentration of glucose

2) Enzyme sample

(Sample) - (Blank)

Calculate glucose concentration according to a standard curve

Activity (SAVIU/ml):

$$\frac{X \text{ (mg glucose/l)} * \text{Dilution}}{180.16 \text{ (MW of glucose)} * 20 \text{ (min)}}$$

C. Purification and characterisation of the endoglucanase from *M. thermophila*

Aspergillus oryzae transformed with pA2C193 was grown on YPM medium for 4 days. The liquid was then centrifuged and sterile filtered.

The sample was concentrated by ultrafiltration on AMICON cells using a DOW membrane GR61PP with cut-off 20 kD. The Uf-concentrate was analyzed for S-CEVU/ml and SaviU/ml with the following result:

UF-concentrate	S-CEVU/ml	SaviU/ml
9.25 ml	570	41

Purification:
2 ml of the UF-concentrate was diluted 5 times to lower the ionic strength and filtered through 0.22 μm disk filter. This sample was applied to a Mono Q® HR5/5 Pharmacia column, equilibrated with 50 mM Tris/HCl buffer, pH 7.5, (buffer A) and a flow of 1 ml/min. After wash to baseline, with buffer A, the column was eluted with a Tris/HCl buffer, pH 7.5, containing 1 M NaCl (buffer B), the elution gradient was 0-50% buffer B in 1 hour.

After 36 min. a peak complex showed up, 1 ml fractions were picked up and the first 10 fractions showed cellulase activity on CMC/Agarose/congo-red plates.

These fractions were pooled and concentrated, by ultrafiltration on AMICON cells using a DOW membrane GR61PP with cut-off 20 kD, to 3 ml.

This sample was applied to a HiLoad 26/60 Superdex 75™ prep grade Pharmacia column, equilibrated with 100 mM Na-Acetate buffer, pH 6.35, and a 1 ml/min flow.

After 82 min. a peak showed up, 1 ml fractions were picked up and the first 10 fractions showed cellulase activity on CMC/Agarose/congo-red plates.

These fractions were pooled and the following results were obtained:

A_{280}=0.15
A_{280}/A_{260}=1.62
Mw(SDS)=22 kD
pI=3.5 - 5
Purity on SDS-PAGE =100%
S-CEVU/ml=28.5
S-CEVU/A_{280}=188
S-CEVU/mg=436
Extinction coefficient=54880 (calculated)
Mw(calculated)=22 kD

The Extinction coefficient is based on the content of tyrosine, tryptophane and cystein calculated from the sequence of the enclosed SEQ ID No. 1 (the amino acid sequence). SDS-Page was performed on NOVEX Pre-Cast Gels 4-20% Tris-Glycine Gel 1.0 mm x 10 Well

IEF was performed on Pharmacia PAGplate pH 3.5 - 9.5, the activity was visualized by CMC-Congored overlaying.

Determination of K_M & k_{cat}:
k_m and k_{cat} was determined in the same manner as the determination of SAVI Units at pH 8.5 with a substrate

concentration up to 8 g/l.

The following results were obtained:

k_{cat} 38 per sec.
k_m 5 g/l,
phosporic acid swollen cellulose, pH 8.5.

Specific activity on CMC at pH 7.5:
436 S-CEVU per mg protein.

D. Determination of pH and temperature profile of the endoglucanase from *M. thermophila*

The pH profile was determined at the following conditions:

Buffers of pH values between 2.5 and 10.0 were made by mixing 0.1M Tri-sodium phosphate with 0.1M citric acid. Purified endoglucanase was diluted to ensure the assay response to be within the linear range of the assay. The substrate was a 0.4% suspension of AZCL-HE-cellulose (MegaZyme) mixed 1:1 with the citrate/phosphate buffer to a final substrate concentration of 0.2% AZCL-HE-cellulose. 1 ml substrate in Eppendorf® 1.5ml polypropylene tubes were added 10 µl of enzyme solution and incubated for 15 minutes in Eppendorf® temperature controlled Thermomixers before heat-inactivation of enzymes for 20 minutes at 95°C in a separate Thermomixer. The tubes were centrifuged and 200 µl of each supernatant was transferred to a well in a 96 well microtiter plate and OD was measured at 620nm in an ELISA reader (Labsystems Multiskan® MCC/340).

For the pH optimum incubations took place at 30°C. For each pH value, three tubes were added enzyme and

incubated before heat-inactivation, whereas one tube (the blank) was added enzyme and heat-inactivated immediately. The mean value of the three incubated samples was calculated and the blank value was substracted.

The following pH profile was determined:

pH	Relative Activity
2.5	<10%
3	<10%
3.5	22%
4	87%
4.5	89%
5	100%
6	94%
6.5	86%
7	78%
7.5	73%
8	68%
8.5	54%
9	31%
10	18%

It is seen that the endoglucanase has more than 60% activity between pH 4.0 and 8.0 and optimal activity at pH 5.0-6.0.

Temperature profile:

The temperature optimum was determined in the same manner at pH 5.5. The temperatures ranged from 30°C to 80°C. For each temperature three incubations were carried out and the mean calculated. Three blanks were produced by immediate heat-inactivation of enzyme and the mean was subtracted from the incubated sample values.

It is seen that the endoglucanase has optimal activity at 50-70°C.

Temp. (°C)	30	40	50	60	70	80
Relative Activity	74%	77%	99%	100%	93%	62%

The temperature stability was determined in the same manner at pH 5.5 and 30°C, and, further, the enzyme solutions were preheated for 1 hour at the actual temperature and cooled on ice. The residual activity is shown below in % of the activity of a non-preheated enzyme sample:

Temp. (°C)	40	50	60	70	80
Relative Activity	95%	84%	92%	86%	24%

E. Color clarification of Myceliophthora cellulase (SEQ ID No. 1) measured as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	100 ml
Agitation stirrer	:	150 movements/min with vertical
Rinse time	:	5 min in tapwater
Washing temp	:	40°
Washing liqour	:	0.05 M phosphate buffer
pH	:	7.0
Washing time	:	30 min
Repetitions	:	2
Enzymes	:	Myceliophthora SEQ ID No. 1B
Dosage	:	500 and 2500 S-CEVU/l
Textile	:	2 swatches of aged black 100% cotton 5x6 cm (0.9 gram)
Drying	:	Tumble dry
Evaluation	:	The light remission is measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker, and lower L values are obtained.

The sample is compared with a blind sample, i.e. washed without enzyme:

No cellulase	500 ECU/l	2500 ECU/l
0.00	-1.41	-1.91

Delta L-values compared to blind sample.

The data shows that Myceliophthora cellulase without CBD gives good color clarification under the conditions tested.

F. Construction of the gene fusions between the endoglucanase from Myceliophthora thermophila and the 43kD endoglucanase from Humicola insolens

The purpose of the two constructions was to make derivatives of the endoglucanase from M. thermophila with the linker and CBD from the 43kD endoglucanase from *H. insolens* (disclosed in WO 91/17243). The native endoglucanase from *M. thermophila* do not have a linker and/or a cellulose binding domain, CBD.

CM1: Construction 1 consist of the endoglucanase from M.thermophila (225 amino acids) and the 72 C-terminal amino acids from the H. insolens 43kD endoglucanase.

CM2: Construction 2 consist of the endoglucanase from M.thermophila (225 amino acids) and the 83 C-terminal amino acids from the H. insolens 43kD endoglucanase.

The 43kD endoglucanase cDNA from H. insolens was cloned into pHD414 in such a way that the endoglucanase gene was transcribed from the Taka-promoter. The resulting plasmid was named pCaHj418.

In a similar way the cDNA encoding the endoglucanase from *M. thermophila* was cloned into pHD414 and the resulting plasmid was named pA2C193.

Primers:

primer 1: 5'-
CGGAGCTCACGTCCAAGAGCGGCTGCTCCCGTCCCTCCAGCAGCACCAGCTCTCCGG
-3'
primer 2: 5'
CCGGAGAGCTGGTGCTGCTGGAGGGACGGGAGCAGCCGCTCTTGGACGTGAGCTCCG
-3'
primer 3: 5'-
CGGAGCTCACGTCCAAGAGCGGCTGCTCCCGTAACGACGACGGCAACTTCCCTGCCG
-3'
primer 4: 5'-
CGGCAGGGAAGTTGCCGTCGTCGTTACGGGAGCAGCCGCTCTTGGACGTGAGCTCCG
-3'
Taka-pro. primer: 5' CAACATCACATCAAGCTCTCC -3'
AMG-term. primer: 5' CCCCATCCTTTAACTATAGCG -3'

The endoglucanase fusions were constructed by the PCR overlap-extension method as described by Higuchi et al. 1988.

Construction 1:

Reaction A: The Polymerase Chain Reaction (PCR) was used to amplify the fragment of pCaHj418 between primer 1 and AMG-term. primer (the linker and CBD from the 43kD endoglucanase from H.insolens).
Reaction B: PCR amplification of the fragment between Taka-pro. primer and primer 2 in pA2C193, the endoglucanase gene from M.thermophila.
Reaction C: The two purified fragments were used in a third PCR in the presence of the primers flanking the total region, i.e. Taka-pro. primer and AMG-term. primer.

Construction 2:

The same procedure was used where primer 3 and primer 4

had replaced respectively primer 1 and primer 2.

The fragment amplified in reaction C was purified, digested with restriction enzymes Xba I and BsstE II. The purified digested fragment was ligated into pA2C193 digested with restriction enzymes Xba I and BsstE II.

Competent cells from E. coli strain DH5αF′ (New England Biolabs.) were transformed with the ligated plasmid and colonies containing the gene fusion were isolated. The sequence of the cloned part was verified by DNA sequencing.

The sequence of the gene in the two constructs are shown in SEQ ID No. 2A and SEQ ID No. 3A.

Polymerase Chain Reactions were carried out under standard conditions, as recommended by Perkin-Elmer.

Reaction A and B started with 2 min. at 94°C followed by 20 cycles of (30 sec. at 94°C, 30 sec. at 50°C and 1 min. at 72°C) and end with 4 min. at 72 °C.

Reaction C started with (2 min. at 94°C, 1 min. at 52°C and 2 min. at 72°C), followed by 15 cycles of (30 sec. at 94°C, 30 sec. at 52°C and 90 sec. at 72°C) and end with 4 min. at 72°C.

The two constructs were transformed into Aspergillus oryzae as described above.

G. Purification and characterisation of cloned cellulases with cellulose binding domains:

The cloned product is recovered after fermentation by separation of the extracellular fluid from the production

organism.
About one gram of cellulase is then highly purified by affinity chromatography using 150 gram of Avicel in a slurry with 20 mm Sodium- phosphate pH 7.5.

The Avicel is mixed with the crude fermentation broth which contain total about 1 gram of cellulase. After mixing at 4 C for 20 min the Avicel enzyme is packed into a column with a dimension of 50 times 200 mm about 400 ml total.

The column is washed with the 200 ml buffer, then washed with 0.5 M NaCl in the same buffer until no more protein elutes. Then washed with 500 ml 20 mm Tris pH 8.5. Finally the pure full length enzyme is eluted with 1% triethylamine pH 11.8.

The eluted enzyme solution is adjusted to pH 8 and concentrated using a Amicon cell unit with a membrane DOW GR61PP (polypropylene with a cut off of 20 KD) to above 5 mg protein per ml.
The purified cellulases were characterised as follow:

	Mw SDS-PAGE	pI	Molar E.280	S-CEVU per A.280
Myceliophthora (SEQ ID No.2)	43 kD	4	74.950	135
Acremonium (SEQ ID No.5)	40 kD	5	68.020	185
Thielavia (SEQ ID No.9)	35 kD	4.3	52.470	75

	pH Activity above 50%	N-terminal	Termo-stability DSC
Myceliophthora (SEQ ID No.2)	5.0-9.0	Blocked.	80°C
Acremonium (SEQ ID No.5)	6.0-9.5	Blocked.	61°C
Thielavia (SEQ ID No.9)	5.0-9.0	ASGSG---	83°C

The purified cellulases was analysed for MW by SDS-PAGE and using standard LMW protein marker kit from Pharmacia the MW was calculated for the cellulases. The MW is apparently higher than the MW of the composition of the coding amin acids and is due to the fact the linker region are O-glycosylated resulting in this higher MW. The pI was determined using a Pharmacia Ampholine PAG plates pH 3.5 to 9.5 and again using a Pharmacia kit with known pI proteins.

The molar extinction coefficient was calculated based on the amin acids composition using the known absorbance of Tryptophan, Tyrosine and Cystein.

pH activity profile was obtained using CMC substrate, incubation for 20 min at 40° C at a 0.5 pH interval and measuring the formation of reducing sugars. The relative activity at the different pH was calculated and the table contain the interval with more than 50% relative activity has been measured.

The N-terminal was determined for the purified cellulase using a Applied Biosystems model 473A sequencer. The protein sequenceer was run according to the manufacturer

instructions.

Two of the cellulases were blocked, this is due to the N-terminal glutamine which form a pyroglutamate which can not be detected and which block for further sequencing. DSC Differential scanning calometry was done at neutral pH (7.0) using a MicroCalc Inc. MC calorimeter with a constant scan rate and raising the temperature from 20 to 90° at a rate of 90° per hour.

Raising antibody. The cellulases from Myceliophthora, Acremonium and Thielavia were used for raising antibody in rabits. 0.1 mg of the purified cellulase in 0.9 % NaCl solution mixed with Freunds adjuvant immediately prior to injection. The rabits were immunized 10 times with one week interval. The immunoglobulin G fraction (IgG) was purified by ammonium sulfate precipitation (25% saturation). the precipitate was solubilized in water and then dialyzed extensively against sodium acetate buffer (pH 5.0, 50 mM) altering with deionized water. After filtration, the IgG fraction was stabilized with sodium azide (0.01%).

Using immunodiffusion in agar plates all three cellulases form a single immunoprecipitate with its homologous antiserum and no precipitate was seen between the 3 cloned cellulases and the sera raised against the other two cellulases.

H-I. Performance of endoglucanase of construction 1 (SEQ ID No. 2) measured in buffer as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	100 ml
Agitation	:	150 movements/min (rpm)
Rinse time	:	5 min in tap water
Washing temp	:	40°C
Water Hardness	:	1 mM $CaCl_2$
Washing liquor	:	0.05 M phosphate buffer
pH	:	7.0
Washing time	:	30 min
Repetitions	:	2
Textile	:	2 swatches of aged black, 100% cotton 5x6 cm
Drying	:	Tumble dry

Evaluation:
The light remission was measured by a Macbeth Color Eye 7000 Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn were removed by the cellulase, the surface appeared more bright, and lower L values were obtained.

Results:

S-CEVU/l	0	250	1000
Inventive enzyme	0	-1.4	-1.6

The data show that the enzyme of the invention gives very good color clarification under the conditions tested.

H-II. Performance of cloned endoglucanase from *Thielavia terrestris* (SEQ ID No.9) in buffer measured as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	100 ml
Agitation	:	150 movements/min with vertical stirrer
Rinse time	:	10 min in tapwater
Washing temp	:	40°
Washing liqour	:	0.05 M phosphate buffer.
pH	:	7.0
Washing time	:	30 min
Repetitions	:	2
Textile	:	2 swatches of aged black cotton 5x6 cm (app. 150 g/m2)
Drying	:	Tumble dry

Evaluation :

The light remission was measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker and nicer, and lower L values are obtained.

Results:

S-CEVU/1	0	50	200
Inventive enzyme	0	-0.66±0.10	-1.32±0.06

The data show that the cellulase gives good color clarification under the conditions tested.

H-III. Performance of endoglucanase of *Volutella colletrichoides* (SEQ ID No. 17) measured in buffer as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	100 ml
Agitation	:	150 movements/min with vertical stirrer
Rinse time	:	5 min in tapwater
Washing temp	:	40°
Washing liqour	:	0.05 M phosphate buffer
pH	:	7.0
Washing time	:	30 min
Repetitions	:	2
Dosage	:	2.5 S-CEVU/ml
Textile	:	2 swatches of aged black 100% cotton 5x6 cm (0.9 gram)
Drying	:	Tumble dry

Evaluation:
The light remission is measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker, and lower L values are obtained.

The sample is compared with a blind sample, i.e. washed without enzyme:

No cellulase	With cellulase
0.00	-0.57

Delta L remission values compared to blind sample.

The data shows that the *Volutella colletrichoides* cellulase gives good color clarification under the conditions tested.

H-IV. Performance of cloned cellulases from *Thielavia terrestris* and *Acremonium sp.* CBS 478.94 in high pH heavy duty detergent measured as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	150 ml
Agitation	:	150 movements/min with vertical stirrer
Rinse time	:	10 min in tapwater
Washing temp	:	35°C
Washing liqour	:	1.0 g/l US type HDG (zeolite/soda built, anionic/nonionic weight ratio > 2.5)
pH	:	10.0
Hardness	:	1.0 mM CaCl2 0.34 mM MgCl2
Washing time	:	12 min
Repetitions	:	6
Textile	:	2 swatches of aged black cotton 5x6 cm (app. 150 g/m2) 2 swatches of heavy knitted cotton 5x6 cm (app. 600 g/m2)
Drying	:	Tumble dry

Evaluation :

The light remission is measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker and nicer, and lower L values are obtained. Different dosages of cloned cellulases from *Thielavia terrestris* (SEQ ID No. 9) and *Acremonium sp.* CBS 478.94 (SEQ ID No.5), respectively, (denoted A and B, respectively) were tested.

Results:

S-CEVU/l	0	500	2000
A	0	-2.09 ±0.22	-2.86 ±0.19
B	0	-0.60 ±0.36	-1.96 ±0.23

The data show that both cellulases gives good color clarification under the conditions tested.

H-V. Performance of cellulases cloned from *Thielavia terrestris* and *Acremonium sp.* CBS 478.94, and construction 1 (SEQ ID No.2) measured as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers

Apparatus	:	Terg-o-tometer
Liquid volume	:	150 ml
Agitation	:	150 movements/min with vertical stirrer
Rinse time	:	10 min in tapwater
Washing temp	:	35°C
Hardness	:	1.0 mM CaCl2 0.34 mM MgCl2
Washing liqour	:	2.0 g/l HDL (neutral, citrate built HDL, with nonionic/anionic weight ration > 0.5)
pH	:	7.5
Washing time	:	30 min
Repetitions	:	2
Textile	:	2 swatches of aged black cotton 5x6 cm (app. 150 g/m2) 2 swatches of heavy knitted cotton 4x7 cm (app. 600 g/m2)

Drying : Tumble dry

Evaluation :

The light remission is measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (CIE Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker and nicer, and lower L values are obtained. Three different dosages Different dosages of cloned cellulases from *Thielavia terrestris* (SEQ ID No. 9) and *Acremonium sp*. CBS 478.94 (SEQ ID No.5) and the construction 1 (SEQ ID No.2), respectively, (denoted A and B and C, respectively) were tested.

Results:

S-CEVU/1	0	100	200	400
A	0	-3.06 ±0.24	-3.15 ±0.27	-3.92 ±0.26
B	0	-1.75 ±0.27	-3.08 ±0.32	-3.51 ±0.44
C	0	-1.84 ±0.39	-1.70 ±0.47	-2.30 ±0.61

The data show that all cellulases gives very good color clarification under the conditions tested.

I. Application of endoglucanases from Thielavia terrestris, Acremonium sp. and construction 1 (SEQ ID No. 2) in denim finishing

Experimental

Apparatus: Washing machine Wascator FL 120

Liquid volume: 20 L

Fabric: 1.1 kg denim fabric, 14½ oz 100 %

	cotton
Desizing:	10 min, 55°C, pH 7
	50 ml Aquazyme 120L
	2.5 g/l Phosphate buffer
Abrasion:	2 hours;

pH and temperature varied according to the following table:

Enzyme SEQ ID	Activity	pH/temp	Buffer system
No. 2	1400 S-CEVU/g	6/55°C	2.5 g/l phosphate buffer
No. 9	292 S-CEVU/g	5/65°C	1 g/l citrate buffer
No. 5	782 S-CEVU/g	7/45°C	2.5 g/l phosphate buffer

Inactivation: 15 min, 80°C
1 g/l sodium carbonate

Rinses: Three rinse cycles of 5 min in cold tap water

Evaluation:

Abrasion: The remission from the fabric was determined at 420 nm using a Texflash 2000 as a measure of the abrasion level.

The results from the treatment of the denim fabric with different endoglucanases of the invention is shown in the following table:

Enzyme	Dosage	Trial conditions	Abrasion 420 nm
Blank	0 S-CEVU/g textile	pH 6, 55°C	9.96
SEQ ID No. 2	10 S-CEVU/g textile	pH 6, 55°C	14.37
Blank	0 S-CEVU/g textile	pH 5, 65°C	9.26
SEQ ID No. 9	10 S-CEVU/g textile	pH 5, 65°C	16.86
Blank	0 S-CEVU/g textile	pH 7, 45°C	9.47
SEQ ID No. 5	10 S-CEVU/g textile	pH 7, 45°C	14.08

All tested cellulases show excellent performance in denim finishing, although each enzyme is unique in its own way. When applying the enzyme corresponding to SEQ ID No. 2 for denim finishing it is possible to reach a high abrasion level with a minimum of strength loss. When treating denim with the enzyme corresponding to SEQ ID No. 9, a very high wash down can be reached which leaves the fabric with an almost bleached appearance. Denim finishing with the enzyme corresponding to SEQ ID No. 5 gives a high abrasion level at a low temperature optimum which makes it possible to reduce the processing temperature and save energy.

J. Use of cloned cellulases from *Acremonium sp.* and *Thielavia terrestris* for Biopolishing of lyocell fibers

Lyocell fibers which are sold under the trade name Tencel

are spun from wood pulp cellulose in a more environmentally friendly waterbased solvent than is the case for normal viscose production). However, the fibers have a tendency to fibrillate when they are processed into textiles which is seen on the surface and denoted "fuzz". By using cellulases it is possible to permanently remove the exposed and fuzzy fibers and significantly improve the look of the finished fabric, the treatment generally known as Biopolishing. The endoglucanases of the present invention are especially suited for the removal of Lyocell surface fibers.

MATERIALS AND METHODS

The textile substrate was either 100 % woven or different kinds of jersey knitted dark blue Tencel. The dark colour and jersey knit was preferred in order to enhance the visual effects which simplifed the evaluation. A woven 70/30 Tencel/Rayon blend was also used to a lesser extent.

The assays were either performed in 200 ml scale using a Launder-o-meter or in the 20 l scale using a Wascator. The treatment time was 60min at 55° C in Wascator and 60-90 min in LOM. The buffer was 2 g/l sodium acetate adjusted to pH 5 with acetic acid. The fabric to liquid ratio was 1:10 but in the Launder-o-meter 20 steel balls with a diameter of 14 mm (11 g each) was used to obtain sufficient mechanical abrasion. The biopolishing was immediately followed by inactivation using 2 g/lit sodium carbonate at 80° C for 15 min followed by rinsing in cold water.

The results were evaluated using a fuzz note scale from 1 - 5 were 1 is the fibrillated look of the starting material and 5 is a high quality look with no visible fibers on the surface. Since the performance of endocellulases is specific towards a surface treatment the weightloss is below 2 % and is therefore not included in the evaluation. Two cellulases were evaluated: the

cellulases cloned from *Acremonium sp.* (SEQ ID No. 5) and from *Thielavia terrestris* (SEQ ID No. 9).

The two cellulases are able to defibrillate both Tencel and Tencel blended fabrics. By using an endoglucanase of the invention, only small fibrils are removed rather than whole fibers such as is the case when using acid cellulase mixtures from *Trichoderma*. The strength loss of the treated fabric is threrefore kept at a minimum when using endoglucanases of the present invention.

The following dosages gave a superior defibrillation, i.e. fuzz note 4 or above:

15 S-CEVU/g fabric of cellulase from *Acremonium sp* (SEQ ID No. 5); and
10 S-CEVU/g fabric of cellulase from *Thelavia terrestris* (SEQ ID No.9).

EXAMPLE 2

A new cellulytic enzyme was by expression cloning as well as by PCR cloning detected to be produced by a plant pathogen, isolated from soy bean seeds and identified as Macrophomina phaseolina.

Production of biomass for PCR and expression cloning procedures:
Isolate CBS 281.96 was grown in shake flask cultures on cellulose enriched potato dextrose broth, incubated for 5 days at 260C (shaking conditions: 150 rpm).

A. Cloning and expression of an endoglucanase from *Macrophomina phaseolina*

mRNA was isolated from *Macrophomina phaseolina*, grown in a cellulose-containing fermentation medium with agitation

to ensure sufficient aeration. Mycelia were harvested after 3-5 days' growth, immediately frozen in liquid nitrogen and stored at -80°C. A library from *Macrophomina phaseolina*, consisting of approx. 10^6 individual clones was constructed in *E. coli* as described with a vector background of 1%.

Plasmid DNA from some of the pools was transformed into yeast, and 50-100 plates containing 250-400 yeast colonies were obtained from each pool.

Endoglucanase-positive colonies were identified and isolated on SC-agar plates with the AZCL HE cellulose assay. cDNA inserts were amplified directly from the yeast colonies and characterized as described in the Materials and Methods section above. The DNA sequence of the cDNA encoding the endoglucanase is shown in SEQ ID No. 10 and the corresponding amino acid sequence is shown in SEQ ID No. 11.

The cDNA is obtainable from the plasmid in DSM 10512.

Total DNA was isolated from a yeast colony and plasmid DNA was rescued by transformation of *E. coli* as described above. In order to express the endoglucanse in *Aspergillus*, the DNA was digested with appropriate restriction enzymes, size fractionated on gel, and a fragment corresponding to the endoglucanase gene was purified. The gene was subsequently ligated to pHD414, digested with appropriate restriction enzymes, resulting in the plasmid pA2C477.

After amplification of the DNA in *E. coli* the plasmid was transformed into *Aspergillus oryzae* as described above.

Screening of the cDNA library by hybridization and characterization f the positiv clones. Approximately

6000 colony forming units (c.f.u.) from the *Macrophomina phaseolina* cDNA library in E. coli was screened by colony hybridization using a random-primed ^{32}P-labeled PCR product from *M. phaseolina* as probe. The PCR product was generated as described in the Materials and methods section. The positive cDNA clones were characterized by sequencing the ends of the cDNA inserts, and by determining the nucleotide seuence of the longest cDNA from both strands. The DNA sequence of the cDNA encoding the endoglucanase is shown in SEQ ID No. 10 and the corresponding amino acid sequence is shown in SEQ ID No. 11.

B. Construction of gene fusion between the endoglucanase from *Macrophomina phaseolina* and the 43 kD endoglucanase from Humicola insolens

One construction was prepared in order to make a derivative of the endoglucanase from *M. phaseolina* with the linker and CBD from the 43 kD endoglucanase from *H. insolens* (disclosed in WO 91/17243). The native endoglucanase from *M. phaseolina* does not have a linker and/or a cellulose binding domain, CBD.

The construction consists of the endoglucanase from *M. phaseolina* (223 amino acids) and the 72 C-terminal amino acids from the *H. insolens* 43 kD endoglucanase.

The 43 kD endoglucanase cDNA from *H. insolens* is cloned into pHD414 in such a way that the endoglucanase gene is transcribed from the Taka-promoter. The resulting plasmid is named pCaHj418.

The cDNA encoding the endoglucanase from *M. phaseolina* is cloned into pYES2.0 as a BstX I/Not I fragment and the r sulting plasmid is named pC1C477.

__Primers:__

primer 1: 5'-
GGTCGCCCGGACTGGCTGTTCCCGTACCCCCTCCAGCAGCACCAGCTCTCCGG -3'
primer 2: 5'
CCGGAGAGCTGGTGCTGCTGGAGGGGGTACGGGAACAGCCAGTCCGGGCGACC -3'
pYES2.0 F.HT primer: 5' CGGACTACTAGCAGCTGTAATACG -3'
AMG-term. primer: 5' CCCCATCCTTTAACTATAGCG -3'

The endoglucanase fusion is constructed by the PCR overlap-extension method as described by Higuchi et al. 1988.

Reaction A: The Polymerase Chain Reaction (PCR) is used to amplify the fragment of pCaHj418 between primer 1 and AMG-term. primer (the linker and CBD from the 43 kD endoglucanase from *H.insolens*).
Reaction B: PCR amplification of the fragment between pYES2.0 F.HT primer and primer 2 in pC1C477, the endoglucanase gene from *M. phaseolina*.
Reaction C: The two purified fragments are used in a third PCR in the presence of the primers flanking the total region, i.e. pYES2.0 F.HT primer and AMG-term. primer.

The fragment amplified in reaction C is purified, digested with restriction enzymes, e.g. Xba I and BamH I. The purified digested fragment is ligated into pHD414 digested with restriction enzymes, e.g. Xba I and BamH I.

Competent cells from *E. coli* strain DH5αF' (New England Biolabs) are transformed with the ligated plasmid and colonies containing the gene fusion are isolated. The sequence of the cloned part was verified by DNA sequencing.

Polymerase Chain Reactions are carried out under standard

conditions, as recommended by Perkin-Elmer.

Reaction A and B start with 2 min. at 94°C followed by 20 cycles of (30 sec. at 94°C, 30 sec. at 52°C and 1 min. at 72°C) and ends with 4 min. at 72 °C.

Reaction C starts with (2 min. at 94°C, 1 min. at 52°C and 2 min. at 72°C), followed by 20 cycles of (30 sec. at 94°C, 30 sec. at 52°C and 90 sec. at 72°C) and ends with 4 min. at 72°C.

The construct may be transformed into *Aspergillus oryzae* as described above.

EXAMPLE 3

Cloning and expression of an endoglucanase from *Acremonium sp.* and *Sordaria fimicola*

Production of biomass for expression cloning procedures: Isolates CBS 478.94 and ATCC 52644, respectively, were grown in shake flask cultures on cellulose enriched potato dextrose broth, incubated for 5 days at 260C (shaking conditions: 150 rpm).

mRNA was isolated from *Acremonium sp.*, CBS 478.94, and *Sordaria fimicola*, ATCC 52644, respectively, grown in a cellulose-containing fermentation medium with agitation to ensure sufficient aeration. Mycelia were harvested after 3-5 days' growth, immediately frozen in liquid nitrogen and stored at -80°C. Libraries from *Acremonium sp.*, and *Sordaria fimicola*, respectively, each consisting of approx. 10^6 individual clones were constructed in *E. coli* as described with a vector background of 1%.

Plasmid DNA from s m of the pools from each library was

transformed into yeast, and 50-100 plates containing 250-400 yeast colonies were obtained from each pool.

Endoglucanase-positive colonies were identified and isolated on SC-agar plates with the AZCL HE cellulose assay. cDNA inserts were amplified directly from the yeast colonies and characterized as described in the Materials and Methods section above.

The DNA sequence of the cDNA encoding the endoglucanase from *Acremonium sp.* is shown in SEQ ID No. 6 and the corresponding amino acid sequence is shown in SEQ ID No.7. The cDNA is obtainable from the plasmid in DSM 10080.

The partial DNA sequence of the cDNA encoding the endoglucanase from *Sordaria fimicola* is shown in SEQ ID No. 19 (Nucleotide sequence of the 5'-end of the cDNA) and the corresponding amino acid sequence is shown in SEQ ID No. 20. The cDNA is obtainable from the plasmid in DSM 10576.

Total DNA was isolated from a yeast colony and plasmid DNA was rescued by transformation of *E. coli* as described above. In order to express the endoglucanase in *Aspergillus*, the DNA was digested with appropriate restriction enzymes, size fractionated on gel, and a fragment corresponding to the endoglucanase gene from *Acremonium sp.* and *Sordaria fimicola*, respectively, was purified. The genes were subsequently ligated to pHD414, digested with appropriate restriction enzymes, resulting in the plasmids pA2C371 and pA2C502, respectively.

After amplification of the DNA in *E. coli* the plasmids were transformed into *Aspergillus oryzae* as described above.

EXAMPLE 4

A. Cloning by PCR an endoglucanase from *Crinipellis scabella*, CBS 280.96

Isolate CBS 280.96 was grown in static flask cultures, holding wheat bran medium (per flask: 300g wheat bran added 450 ml salt solution), incubated for 6 days at 26C. After incubation the wheat bran was extracted with destilled water (300ml per flask) and the extract tested for endoglucanase activity (0.1% AZCL-HE-Cellulose (megazyme) in 1% agarose (Litex agarose, Medinova). Activity was observed on the plates holding pH of 3.0, 7.0 and 9.5.

mRNA was isolated from Crinipellis scabella grown as describe above. Mycelia were harvested after 3-5 days' growth, immediately frozen in liquid nitrogen and stored at -80°C. A library from *Crinipellis scabella*, consisting of approx. 10^6 individual clones was constructed in *E. coli* as described with a vector background of 1%.

Approximately 10 000 colony forming units (c.f.u.) from the *Crinipellis scabella* cDNA library in E. coli was screened by colony hybridization using a random-primed ^{32}P-labeled PCR product from *C. scabella* as probe. The PCR product was generated as described in the Materials and methods section. The positive cDNA clones were characterized by sequencing the ends of the cDNA inserts, and by determining the nucleotide seuence of the longest cDNA from both strands.

The DNA sequence of the cDNA encoding the endoglucanase is shown in SEQ ID No. 12 and the corresponding amino acid sequence is shown in SEQ ID No. 13.

The cDNA is obtainable from the plasmid in DSM 10511.

Total DNA was isolated from a yeast colony and plasmid DNA was rescued by transformation of *E. coli* as described above. In order to express the endoglucanse in *Aspergillus*, the DNA was digested with appropriate restriction enzymes, size fractionated on gel, and a fragment corresponding to the endoglucanase gene was purified. The gene was subsequently ligated to pHD414, digested with appropriate restriction enzymes, resulting in the plasmid pA2C475.

After amplification of the DNA in *E. coli* the plasmid was transformed into *Aspergillus oryzae* as described above.

Construction of two gene fusions between the endoglucanase from *Crinipellis scabella* and the linker/CBD region of the 43 kDa endoglucanase from *Humicola insolens*.

The native endoglucanase from *Crinipellis scabella* neither has a linker nor a cellulose binding domain (CBD). In addition, the full-length cDNA contains an ATG start codon upstream from the endoglucanase encoding open reading frame (ORF), presumably resulting in scrambled translation initiation upon heterologous expression of the cDNA, such as in the yeast *Saccharomyces cerevisiae* and the filamentous fungus *Aspergillus oryzae*. Thus, two gene fusions between the endoglucanase from *Crinipellis scabella* and the linker/CBD region of the 43kD endoglucanase from *Humicola insolens* (disclosed in WO 91/17243) has been constructed using splicing by overlap extension (SOE) (Horton et al, 1989).

Construction 1 consists of the cDNA encoding the 226-residue endoglucanase from *C. scabella* fused by PCR with the 3'-end cDNA of *H. insolens* coding for the linker and CBD region (72 amino acids) at the COOH-terminus of the

H. insolens 43kD endoglucanase. The second hybrid construct is identical to the abovementioned gene fusion, except that the first five residues from the putative signal peptide have been deleted by PCR resulting in a shorter signal, which starts with the second in-frame ATG start codon.

Plasmid constructs

The plasmid pC1C475 contains the full-length cDNA from *C. scabella*, cloned into BstXI/NotI-cut yeast expression vector pYES 2.0, the plasmid pC1C144 contains the full-length cDNA from *H. insolens*, cloned into the BstXI site of pYES 2.0.

Splicing by overlap extension

Two PCR fragments encoding the core region of the endoglucanase from *C. scabella* were generated in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin; containing 200 μM each dNTP), using 50-100 ng of pC1C475 as template, and 250 pmol of the reverse primer (5'-GACCGGAGAGCTGGTGCTGCTGGAGGGTTTACGAACACAGCCCGAGATATTAGTG- 3') in two combinations with 300-350 pmol of each forward primer (forward no. 1 5'-CCCCAAGCTTGACTTGGAACCAATGGTCCATCC-3', forward no. 2 5'-CCCCAAGCTTCCATCCAAACATGCTTAAAACGCTCG- 3'), a DNA thermal cycler (Landgraf, Germany) and 2.5 units of Taq polymerase (Perkin-Elmer, Cetus, USA). Thirty cycles of PCR were performed using a cycle profile of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min. The PCR fragment coding for the linker and CBD of the endoglucanase of *H. insolens* was generated in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin; containing 200 μM each dNTP) using 100 ng of the pC1C144 template, 250 pmol

forward primer (5'-CACTAATATCTCGGGC-TGTGTTCGTAAACCCTCCAGCAGCACCA-GCTCTCCGGTC-3'), 250 pmol of the pYES 2.0 reverse primer (5'-GGGCGTGAATGTAAGCGTGACATA-3') a DNA thermal cycler (Landgraf, Germany) and 2.5 units of Taq polymerase (Perkin-Elmer, USA). Thirty cycles of PCR were performed as above. The PCR products were electrophoresed in 0.7 % low gelling temperature agarose gels (SeaPlaque, FMC), the fragments of interest were excised from the gel and recovered by treatment with agarase (New England Biolabs, USA) according to the manufacturer's instructions, followed by phenol extraction and ethanol precipitation at - 20 °C for 12 h by adding 2 vols of 96 % EtOH and 0.1 vol of 3M NaAc.

The recombinant hybrid genes between the endoglucanase from *Crinipellis scabella* and the linker/CBD region of the 43 kD endoglucanase from *Humicola insolens* were generated by combining the overlapping PCR fragments from above (ca. 50 ng of each template) in two combinations in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin; containing 200 μM each dNTP). The SOE reaction was carried out using the DNA thermal cycler (Landgraf, Germany) and 2.5 units of Taq polymerase (Perkin-Elmer, Cetus, USA). Two cycles of PCR were performed using a cycle profile of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min, the reaction was stopped, 250 pmol of each end-primer (forward no. 1 5'-CCCCAAGCTTGACTTGGAACCAATGGTCCATCC-3', forward no. 2 5'-CCCCAAGCTTCCATCCAAACATGCTTAAAACGCTCG-3', reverse primer 5'-GGGCGTGAATGTAAGCGTGACATA-3') was added to the reaction mixture, and an additional 30 cycles of PCR were performed using a cycle profile of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 3 min.

Construction of the expression cassettes for heterologous expression in Aspergillus oryzae

The PCR-generated, recombinant fragments were electrophoresed in a 0.7 % low gelling temperature agarose gel (SeaPlaque, FMC), the fragments of interest were excised from the gel and recovered by treatment with agarase (New England Biolabs, USA) according to the manufacturer's instructions, followed by phenol extraction and ethanol precipitation at - 20 °C for 12 h. The DNA fragments were digested to completion with HindIII and XbaI, and ligated into *Hind*III/*Xba*I-cleaved pHD414 vector followed by electroporation of the constructs into *E. coli* DH10B cells according to the manufacturer's instructions (Life Technologies, USA).

The nucleotide sequence of the resulting gene fusions were determined from both strands as described in the Materials and methods section, SEQ ID no. 14A and 15A. The constructs may be transformed into *A. oryzae* as described.

EXAMPLE 5

PCR facilitated detection of said type of cellulytic enzyme from 46 filamentous and monocentric fungi, representing 32 genera, from 23 families, belonging to 15 orders of 7 classes, covering all in all all four groups of the true Fungi: Ascomycetous, Basidiomycetous, Chytridiomycetous and Zygomycetous fungi

5.1 Materials

1.Diplodia gossypina Cooke
Deposit of Strain, Acc No: CBS 274.96

2.Ulospora bilgramii (Hawksw. et al.) Hawksw. et al.
Acc No of strain: NKBC 1444,

3. Microsphaeropsis sp

4. Ascobolus stictoideus Speg.
Acc No of strain: Q026 (Novo Nordisk collection)
Isolated from goose dung, Svalbard, Norway

5. Saccobolus dilutellus (Fuck) Sacc.
Deposit of strain: Acc No CBS 275.96

6. Penicillium verruculosum Peyronel
Ex on Acc No of species: ATCC 62396

7. Penicillium chrysogenum Thom
Acc No of Strain: ATCC 9480

8. Thermomyces verrucosus Pugh et al
Deposit of Strain, Acc No.: CBS 285.96

9. Xylaria hypoxylon L. ex Greville
Deposit of Strain, Acc No: CBS 284.96

10. Poronia punctata (Fr.ex L.) Fr.
Ref:A.Munk: Danish Pyrenomycetes,
Dansk Botanisk Arkiv, Vol17,1 1957

11. Nodulisporum sp
Isolated from leaf of Camellia reticulatá (Theaceae, Guttiferales),
Kunming Botanical Garden, Yunnan Province, China

12. Cylindrocarpon sp
Isolated from marine sample, the Bahamas

13. Fusarium anguioides Sherbakoff
Acc No of strain: IFO 4467

14. Fusarium poae (Peck) Wr.
Ex on Acc No of species: ATCC 60883

15. Fusarium solani (Mart.)Sacc.emnd.Snyd & Hans.
Acc No of strain: IMI 107.511

16. Fusarium oxysporum ssp lycopersici (Sacc.)Snyd. & Hans.
Acc No of strain: CBS 645.78

17. Fusarium oxysporum ssp passiflora
Acc No of strain: CBS 744.79

18. Gliocladium catenulatum Gillman & Abbott
Acc. No. of strain: ATCC 10523

19. Nectria pinea Dingley
Deposit of Strain, Acc. No. CBS 279.96

20. Sordaria macrospora Auerswald
Ex on Acc No of species: ATCC 60255

21. Humicola grisea Traeen
ex on Acc No for the species: ATCC 22726

22. Humicola nigrescens Omvik
Acc No of strain: CBS 819.73

23. Scytalidium thermophilum (Cooney et Emerson) Austwick
Acc No of strain: ATCC 28085

24. Thielavia thermophila Fergus et Sinden
(syn Corynascus thermophilus)
Acc No of strain: CBS 174.70, IMI 145.136

25. Cladorrhinum foecundissimum Saccardo et Marchal
Ex on Acc No of species: ATCC 62373

26. Syspastospora boninensis
Acc No of strain: NKBC 1515 (Nippon University, profe Tubaki Collection)

27. Chaetomium cuniculorum Fuckel
Acc. No. of strain: CBS 799.83

28. Chaetomium brasiliense Batista et Potual
Acc No of strain: CBS 122.65

29. Chaetomium murorum Corda
Acc No of strain: CBS 163.52

30. Chaetomium virescens (von Arx) Udagawa
Acc.No. of strain: CBS 547.75

31. Nigrospora sp
Deposit of strain, Acc No: CBS 272.96

32. Nigrospora sp
Isolated from:

33. Diaporthe syngenesia
Deposit of strain, Acc No: CBS 278.96

34. Colletotrichum lagenarium (Passerini) Ellis et Halsted
syn Glomerella cingulata var orbiculare Jenkins et Winstead
Ex on acc No of species: ATCC 52609

35. Exidia glandulosa Fr.
Deposit of Strain, Acc No: CBS 277.96

36. Fomes fomentarius (L.) Fr.
Deposit of strain: Acc No. CBS 276.96

37. Spongipellis (?)
Deposit of Strain: Acc No CBS 283.96

38. Rhizophlyctis rosea (de Bary & Wor) Fischer
Deposit of Strain: Acc No.: CBS 282.96

39. Rhizomucor pusillus (Lindt) Schipper
syn: Mucor pusillus
Acc No of strain: IFO 4578

40. Phycomyces nitens (Kunze) van Tieghem & Le Monnier
Acc No of strain: IFO 4814

41 Chaetostylum fresenii van Tieghem & Le Monnier
syn. Helicostylum fresenii
Acc No of strain NRRL 2305

42. Trichothecium roseum, Acc No of strain: IFO 5372

43. Coniothecium sp
Endophyte, isolated from leaf of flowering plant, Kunming , Yunnan, China

44. Deposit of strain, Acc No.: CBS 271.96
Coelomycete, Isolated from leaf of Artocarpus altilis (Moraceae, Urticales), Christiana, Jamaica

45. Deposit of strain, Acc No.: CBS 273.96
Coelomycete, isolated from leaf of Pimenta dioica (Myrtaceae, Myrtales), Dallas Mountain, Jamaica

46. Deposit of strain: CBS 270.96
Coelomycete, isolated from leaf of Pseudocalymma alliaceum (Bignoniaceae, Solanales) growing in Dallas Mountain, Jamaica

5.2 Procedure

Maintenance of strains and production of biomass:
The strains were maintained on agar in petrie dishes (9cm) or on slants (see list of Media: PCA and PDA). 44 of the strains were grown in shake flasks under the following growth conditions: general fungal media as PC, PD and PB 9 or YPG (see list of media); incubation time from 3 to 9 days; temperature 26^{0}C; rpm between 150 and 175 . Strain No 14 (F.poae) was grown on wheat bran for 15 days (26^{0}C; static). Strain No 38 was grown in dilute salt solution (DS/2), added 1 cm^2 pieces of autoclaved filter paper.

Activity test:
Activity was tested on 0.1% AZCL-HE-Cellulose (Megazyme) plates (14 cm Petrie dishes), made up in 1% agarose (HSB, Litex Agarose, Medinova). All tests were done in triplicate, viz. AZCL-HE-Cellulose dissolved in three buffers, adjusted to pH 3, 7 or 9.5 (using various proportions of the following two ingredients Citric acid monohydrat, Merck art. No 100244 (21.0 g) dissolved in water, making a total of 1000 ml; 0.1M tri-Sodium dodecabrohydrate, Merck art.no. 6578 (38 g), dissolved in water, making a total of 1000 ml. The mixing is done immidiately before use.

Harvesting of Biomass:
The biomass was harvested by filtering (mesh adjusted to the growth of the fungus, the finest used for fungi which have highly sporulating mycelium as e.g. Fusarium spp.). The biomass on the filter was scraped into a sterile plastic bag and immidiately frozen (by submerging into liquid nitrogen).

5.3 Results

I. Using the PCR screening and amplification techniques described in Materials and Methods the following partial cDNA sequences were obtained:

Saccobolus dilutellus (Fuck) Sacc., CBS 275.96: SEQ ID No. 21 (and the deduced amino acid sequence in SEQ ID No. 22);

Thermomyces verrucosus, CBS 285.96: SEQ ID No. 23 (and the deduced amino acid sequence in SEQ ID No. 24);

Xylaria hypoxylon, CBS 284.96: SEQ ID No. 25 (and the deduced amino acid sequence in SEQ ID No. 26);

Fusarium oxysporum ssp lycopersici, CBS 645.78: SEQ ID No. 27 (and the deduced amino acid sequence in SEQ ID No. 28);

Nectria pinea, CBS 279.96: SEQ ID No. 29 (and the deduced amino acid sequence in SEQ ID No. 30);

Humicola grisea, ATCC 22726: SEQ ID No. 31 (and the deduced amino acid sequence in SEQ ID No. 32);

Humicola nigrescens, CBS 819.73: SEQ ID No. 33 (and the deduced amino acid sequence in SEQ ID No. 34);

Cladorrhinum foecundissimum, ATCC 62373: SEQ ID No. 35 (and the deduced amino acid sequence in SEQ ID No. 36);

Syspastospora boninensis, NKBC 1515: SEQ ID No. 37 (and the deduced amino acid sequence in SEQ ID No. 38);

Nigrospora sp., CBS 272.96: SEQ ID No. 39 (and the deduced amino acid sequence in SEQ ID No. 40);

Chaetostylum fresenii: SEQ ID No. 41 (and the deduced amino acid sequence in SEQ ID No. 42);

Exidia glandulosa, CBS 277.96: SEQ ID No. 43 (and the deduced amino acid sequence in SEQ ID No. 44);

Coniothecium sp.: SEQ ID No. 45 (and the deduced amino acid sequence in SEQ ID No. 46);

Deposition No. CBS 271.96: SEQ ID No. 47 (and the deduced amino acid sequence in SEQ ID No. 48);

Deposition No. CBS 270.96: SEQ ID No. 49 (and the deduced amino acid sequence in SEQ ID No. 50);

Diplodia gossypina, CBS 274.96: SEQ ID No. 51 (and the deduced amino acid sequence in SEQ ID No. 52);
Ulospora bilgramii, NKBC 1444: SEQ ID No. 53 (and the deduced amino acid sequence in SEQ ID No. 54);
Penicillium verruculosum, ATCC 62396: SEQ ID No. 55 (and the deduced amino acid sequence in SEQ ID No. 56);
Poronia punctata: SEQ ID No. 57 (and the deduced amino acid sequence in SEQ ID No. 58);
Fusarium anguioides, IFO 4467: SEQ ID No. 59 (and the deduced amino acid sequence in SEQ ID No. 60);
Thielavia thermophila, CBS 174.70: SEQ ID No. 61 (and the deduced amino acid sequence in SEQ ID No. 62);
Chaetomium cuniculorum, CBS 799.83: SEQ ID No. 63 (and the deduced amino acid sequence in SEQ ID No. 64);
Chaetomium virescens: SEQ ID No. 65 (and the deduced amino acid sequence in SEQ ID No. 66);
Colletotrichum lagenarium: SEQ ID No. 67 (and the deduced amino acid sequence in SEQ ID No. 68);
Phycomyces nitens: SEQ ID No. 69 (and the deduced amino acid sequence in SEQ ID No. 70); and
Trichothecium roseum: SEQ ID No. 71 (and the deduced amino acid sequence in SEQ ID No. 72);

II. Using the PCR screening and amplification techniques described in Materials and Methods partial cDNA encoding partially for the enzyme of the invention was obtained and the plasmid was deposited according to the Budapest Treaty:

Escherichia coli, DSM 10583, deposition date 13 March, 1996;
cDNA from Trichothecium roseum;
Escherichia coli, DSM 10584, deposition date 13 March, 1996;
cDNA from Syspastospora boninensis;
Escherichia coli, DSM 10585, deposition date 13 March, 1996;
cDNA from Cheatomium murorum

Escherichia coli, DSM 10587, deposition date 13 March, 1996;
cDNA from Sordaria fimicola;
Escherichia coli, DSM 10588, deposition date 13 March, 1996;
cDNA from the unidentified strain CBS 273.96;
Escherichia coli, DSM 10586, deposition date 13 March, 1996;
cDNA from Spongipellis sp.

Color clarification of crude supernatants

During normal wash the fabric will often fade. However, the fabric appearance is improved and the original colours are much better preserved or maintained if the fabric is washed with a cellulase giving color clarification. Color clarification is measured as removal of surface fibrils and fibers protruding from the yarn of a textile containing cellulosic fibers.

Apparatus	:	Terg-o-tometer
Liquid volume	:	100 ml
Agitation	:	150 movements/min with vertical stirrer
Rinse time	:	5 min in tapwater
Washing temp	:	40°
Washing liqour	:	0.05 M phosphate buffer
pH	:	7.0
Washing time	:	30 min
Repetitions	:	2
Enzymes	:	Crude supernatants from the strains shown below.
Dosage	:	Two dosages from : 200, 500, 1000 or 2500 S-CEVU/l
Textile cotton	:	2 swatches of aged black 100% 5x6 cm (0.9 gram)

Drying : Tumble dry

Evaluation:

The light remission is measured by a Datacolor Elrepho Remission spectrophotometer. Remission is calculated as delta L (Hunter Lab-values). When the surface fibrils and fibers protruding from the yarn are removed by the cellulase, the surface of the black fabric appears darker, and lower L values are obtained.

The samples are compared with a blind sample, i.e. washed without enzyme. Below is shown the delta L remission values compared to a blind sample.

		ECU/l			
Strain		200	500	1000	2500
13.	Fusarium anguioides	n.t.	-0.71	n.t.	-1.28
15.	Fusarium solani	n.t.	-0.96	n.t.	-1.37
24.	Thielavia thermophila	-0.30	n.t.	-1.25	n.t.
25.	Cladorrhinum foecun.	n.t.	-1.79	n.t.	-2.18
37.	Spongipellis (?)	n.t.	-1.01	n.t.	-1.63
39.	Rhizomucor pusillus	n.t.	-1.90	n.t.	-2.66
41.	Chaetostylum fresenii	n.t.	-0.17	n.t.	-1.33
45.	Acc No.: CBS 273.96	n.t.	-1.31	n.t.	-1.20

The data shows that all strains gives good color clarification under the conditions tested. (n.t. = not tested at this dosage).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Novo Nordisk A/S
(B) STREET: Novo Alle
(C) CITY: DK-2880 Bagsvaerd
(E) COUNTRY: Denmark
(F) POSTAL CODE (ZIP): DK-2880
(G) TELEPHONE: +45 44 44 88 88
(H) TELEFAX: +45 44 49 32 56

(ii) TITLE OF INVENTION: NOVEL ENDOGLUCANASES

(iii) NUMBER OF SEQUENCES: 72

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 891 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae
(B) STRAIN: DSM 9770
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1A:

AAAGAAAGGC TCTCTGCTGT CGTCGCTCTC GTCGCTCTCG TCGGCATCCT CCATCCGTCC 60

GCCTTTGATA ACCCGCTCCC CGACTCAGTC AAGACGACGC ATACTTGGCA CCATGCATCT 120

CTCCGCCACC ACCGGGTTCC TCGCCCTCCC GGTCCTGGCC CTGGACCAGC TCTCGGGCAT 180

CGGCCAGACG ACCCGGTACT GGGACTGCTG CAAGCCGAGC TGCGCCTGGC CCGGCAAGGG 240

CCCCTCGTCT CCGGTGCAGG CCTGCGACAA GAACGACAAC CCGCTCAACG ACGGCGGCTC 300

CACCCGGTCC GGCTGCGACG CGGGCGGCAG CGCCTACATG TGCTCCTCCC AGAGCCCCTG 360

GGCCGTCAGC GACGAGCTGT CGTACGGCTG GGCGGCCGTC AAGCTCGCCG GCAGCTCCGA 420

GTCGCAGTGG TGCTGCGCCT GCTACGAGCT GACCTTCACC AGCGGGCCGG TCGCGGGCAA 480

GAAGATGATT GTGCAGGCGA CCAACACCGG TGGCGACCTG GGCGACAACC ACTTTGACCT 540

GGCCATCCCC GGTGGCGGTG TCGGTATTTT CAACGCCTGC ACCGACCAGT ACGGCGCTCC 600

CCCGAACGGC TGGGGCGACC GCTACGGCGG CATCCATTCC AAGGAAGAGT GCGAATCCTT 660

CCCGGAGGCC CTCAAGCCCG GCTGCAACTG GCGCTTCGAC TGGTTCCAAA ACGCCGACAA 720

CCCGTCGGTC ACCTTCCAGG AGGTGGCCTG CCCGTCGGAG CTCACGTCCA AGAGCGGCTG 780

CTCCCGTTAA GAGGGAAGAG AGGGGGCTGG AAGGACCGAA AGATTCAACC TCTGCTCCTG 840

CTGGGGAAGC TCGGGCGCGA GTGTGAAACT GGTGTAAATA TTGTGGCACA CACAAGCTAC 900

TACAGTCCGT CTCGCCGTCC GGCTAACTAG CCTTGCTGCG GATCTGTCCA AAAAAAAAAA 960

(2) INFORMATION FOR SEQ ID NO: 1B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1B:

Met His Leu Ser Ala Thr Thr Gly Phe Leu Ala Leu Pro Val Leu Ala
1 5 10 15

Leu Asp Gln Leu Ser Gly Ile Gly Gln Thr Thr Arg Tyr Trp Asp Cys
20 25 30

Cys Lys Pro Ser Cys Ala Trp Pro Gly Lys Gly Pro Ser Ser Pro Val

35 40 45

Gln Ala Cys Asp Lys Asn Asp Asn Pro Leu Asn Asp Gly Gly Ser Thr

50 55 60

Arg Ser Gly Cys Asp Ala Gly Gly Ser Ala Tyr Met Cys Ser Ser Gln

65 70 75 80

Ser Pro Trp Ala Val Ser Asp Glu Leu Ser Tyr Gly Trp Ala Ala Val

85 90 95

Lys Leu Ala Gly Ser Ser Glu Ser Gln Trp Cys Cys Ala Cys Tyr Glu

100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Ile Val Gln

115 120 125

Ala Thr Asn Thr Gly Gly Asp Leu Gly Asp Asn His Phe Asp Leu Ala

130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asn Ala Cys Thr Asp Gln Tyr

145 150 155 160

Gly Ala Pro Pro Asn Gly Trp Gly Asp Arg Tyr Gly Gly Ile His Ser

165 170 175

Lys Glu Glu Cys Glu Ser Phe Pro Glu Ala Leu Lys Pro Gly Cys Asn

180 185 190

Trp Arg Phe Asp Trp Phe Gln Asn Ala Asp Asn Pro Ser Val Thr Phe

195 200 205

Gln Glu Val Ala Cys Pro Ser Glu Leu Thr Ser Lys Ser Gly Cys Ser

210 215 220

Arg
225

(2) INFORMATION FOR SEQ ID NO: 2A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 894 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: "Construction 1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2A:

ATGCATCTCT CCGCCACCAC CGGGTTCCTC GCCCTCCCGG TCCTGGCCCT GGACCAGCTC
60

TCGGGCATCG GCCAGACGAC CCGGTACTGG GACTGCTGCA AGCCGAGCTG CGCCTGGCCC
120

GGCAAGGGCC CCTCGTCTCC GGTGCAGGCC TGCGACAAGA ACGACAACCC GCTCAACGAC

180

GGCGGCTCCA CCCGGTCCGG CTGCGACGCG GGCGGCAGCG CCTACATGTG CTCCTCCCAG
240

AGCCCCTGGG CCGTCAGCGA CGAGCTGTCG TACGGCTGGG CGGCCGTCAA GCTCGCCGGC
300

AGCTCCGAGT CGCAGTGGTG CTGCGCCTGC TACGAGCTGA CCTTCACCAG CGGGCCGGTC
360

GCGGGCAAGA AGATGATTGT GCAGGCGACC AACACCGGTG GCGACCTGGG CGACAACCAC
420

TTTGACCTGG CCATCCCCGG TGGCGGTGTC GGTATTTTCA ACGCCTGCAC CGACCAGTAC
480

GGCGCTCCCC CGAACGGCTG GGGCGACCGC TACGGCGGCA TCCATTCCAA GGAAGAGTGC
540

GAATCCTTCC CGGAGGCCCT CAAGCCCGGC TGCAACTGGC GCTTCGACTG GTTCCAAAAC
600

GCCGACAACC CGTCGGTCAC CTTCCAGGAG GTGGCCTGCC CGTCGGAGCT CACGTCCAAG
660

AGCGGCTGCT CCCGTCCCTC CAGCAGCACC AGCTCTCCGG TCAACCAGCC TACCAGCACC
720

AGCACCACGT CCACCTCCAC CACCTCGAGC CCGCCAGTCC AGCCTACGAC TCCCAGCGGC
780

TGCACTGCTG AGAGGTGGGC TCAGTGCGGC GGCAATGGCT GGAGCGGCTG CACCACCTGC
840

GTCGCTGGCA GCACTTGCAC GAAGATTAAT GACTGGTACC ATCAGTGCCT GTAG
894

(2) INFORMATION FOR SEQ ID NO: 2B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2B:

Met His Leu Ser Ala Thr Thr Gly Phe Leu Ala Leu Pro Val Leu Ala
1 5 10 15

Leu Asp Gln Leu Ser Gly Ile Gly Gln Thr Thr Arg Tyr Trp Asp Cys
20 25 30

Cys Lys Pro Ser Cys Ala Trp Pro Gly Lys Gly Pro Ser Ser Pro Val
35 40 45

Gln Ala Cys Asp Lys Asn Asp Asn Pro Leu Asn Asp Gly Gly Ser Thr
50 55 60

Arg Ser Gly Cys Asp Ala Gly Gly Ser Ala Tyr Met Cys Ser Ser Gln
65 70 75 80

Ser Pro Trp Ala Val Ser Asp Glu Leu Ser Tyr Gly Trp Ala Ala Val
85 90 95

Lys Leu Ala Gly Ser Ser Glu Ser Gln Trp Cys Cys Ala Cys Tyr Glu
100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Ile Val Gln
115 120 125

Ala Thr Asn Thr Gly Gly Asp Leu Gly Asp Asn His Phe Asp Leu Ala
130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asn Ala Cys Thr Asp Gln Tyr
145 150 155 160

Gly Ala Pro Pro Asn Gly Trp Gly Asp Arg Tyr Gly Gly Ile His Ser
165 170 175

Lys Glu Glu Cys Glu Ser Phe Pro Glu Ala Leu Lys Pro Gly Cys Asn
180 185 190

Trp Arg Phe Asp Trp Phe Gln Asn Ala Asp Asn Pro Ser Val Thr Phe
195 200 205

Gln Glu Val Ala Cys Pro Ser Glu Leu Thr Ser Lys Ser Gly Cys Ser
210 215 220

Arg Pro Ser Ser Ser Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr
225 230 235 240

Ser Thr Thr Ser Thr Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr
245 250 255

Thr Pro Ser Gly Cys Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn
260 265 270

Gly Trp Ser Gly Cys Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys
275 280 285

Ile Asn Asp Trp Tyr His Gln Cys Leu
290 295

(2) INFORMATION FOR SEQ ID NO: 3A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 927 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: "Construction 2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3A:

ATGCATCTCT CCGCCACCAC CGGGTTCCTC GCCCTCCCGG TCCTGGCCCT GGACCAGCTC
60

TCGGGCATCG GCCAGACGAC CCGGTACTGG GACTGCTGCA AGCCGAGCTG CGCCTGGCCC
120

GGCAAGGGCC CCTCGTCTCC GGTGCAGGCC TGCGACAAGA ACGACAACCC GCTCAACGAC

180

GGCGGCTCCA CCCGGTCCGG CTGCGACGCG GGCGGCAGCG CCTACATGTG CTCCTCCCAG
240

AGCCCCTGGG CCGTCAGCGA CGAGCTGTCG TACGGCTGGG CGGCCGTCAA GCTCGCCGGC
300

AGCTCCGAGT CGCAGTGGTG CTGCGCCTGC TACGAGCTGA CCTTCACCAG CGGGCCGGTC
360

GCGGGCAAGA AGATGATTGT GCAGGCGACC AACACCGGTG GCGACCTGGG CGACAACCAC
420

TTTGACCTGG CCATCCCCGG TGGCGGTGTC GGTATTTTCA ACGCCTGCAC CGACCAGTAC
480

GGCGCTCCCC CGAACGGCTG GGGCGACCGC TACGGCGGCA TCCATTCCAA GGAAGAGTGC
540

GAATCCTTCC CGGAGGCCCT CAAGCCCGGC TGCAACTGGC GCTTCGACTG GTTCCAAAAC
600

GCCGACAACC CGTCGGTCAC CTTCCAGGAG GTGGCCTGCC CGTCGGAGCT CACGTCCAAG
660

AGCGGCTGCT CCCGTAACGA CGACGGCAAC TTCCCTGCCG TCCAGATCCC CTCCAGCAGC
720

ACCAGCTCTC CGGTCAACCA GCCTACCAGC ACCAGCACCA CGTCCACCTC CACCACCTCG
780

AGCCCGCCAG TCCAGCCTAC GACTCCCAGC GGCTGCACTG CTGAGAGGTG GGCTCAGTGC
840

GGCGGCAATG GCTGGAGCGG CTGCACCACC TGCGTCGCTG GCAGCACTTG CACGAAGATT
900

AATGACTGGT ACCATCAGTG CCTGTAG
927

(2) INFORMATION FOR SEQ ID NO: 3B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 308 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3B:

```
Met His Leu Ser Ala Thr Thr Gly Phe Leu Ala Leu Pro Val Leu Ala
1               5                   10                  15

Leu Asp Gln Leu Ser Gly Ile Gly Gln Thr Thr Arg Tyr Trp Asp Cys
            20                  25                  30

Cys Lys Pro Ser Cys Ala Trp Pro Gly Lys Gly Pro Ser Ser Pro Val
        35                  40                  45

Gln Ala Cys Asp Lys Asn Asp Asn Pro Leu Asn Asp Gly Gly Ser Thr
    50                  55                  60

Arg Ser Gly Cys Asp Ala Gly Gly Ser Ala Tyr Met Cys Ser Ser Gln
65                  70                  75                  80

Ser Pro Trp Ala Val Ser Asp Glu Leu Ser Tyr Gly Trp Ala Ala Val
```

85 90 95

Lys Leu Ala Gly Ser Ser Glu Ser Gln Trp Cys Cys Ala Cys Tyr Glu

100 105 110

Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Ile Val Gln

115 120 125

Ala Thr Asn Thr Gly Gly Asp Leu Gly Asp Asn His Phe Asp Leu Ala

130 135 140

Ile Pro Gly Gly Gly Val Gly Ile Phe Asn Ala Cys Thr Asp Gln Tyr

145 150 155 160

Gly Ala Pro Pro Asn Gly Trp Gly Asp Arg Tyr Gly Gly Ile His Ser

165 170 175

Lys Glu Glu Cys Glu Ser Phe Pro Glu Ala Leu Lys Pro Gly Cys Asn

180 185 190

Trp Arg Phe Asp Trp Phe Gln Asn Ala Asp Asn Pro Ser Val Thr Phe

195 200 205

Gln Glu Val Ala Cys Pro Ser Glu Leu Thr Ser Lys Ser Gly Cys Ser

210 215 220

Arg Asn Asp Asp Gly Asn Phe Pro Ala Val Gln Ile Pro Ser Ser Ser

225 230 235 240

Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser Thr

245 250 255

Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly Cys

260 265 270

Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly Cys

275 280 285

Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp Tyr

290 295 300

His Gln Cys Leu
305

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 888 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae, DSM 10082

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCAGTGTGCT GGAAAGCCTT CGTGCTGTCC CCGACGTATC CCTGACCGCC ATGCGTTCCA 60

CCAGCATCTT GATCGGCCTT GTTGCCGGCG TCGCTGCTCA GAGCTCTGGC TCTGGCCATA 120

CAACCAGGTA CTGGGACTGC TGCAAGCCCT CATGCGCCTG GGATGAGAAG GCGGCTGTCA 180

GCCGGCCGGT CACAACATGC GACAGGAACA ACAGCCCCCT TTCGCCCGGC GCTGTGAGCG 240

GCTGCGACCC CAACGGCGTT GCATTCACCT GCAACGACAA CCAGCCTTGG GCCGTAAACA 300

ACAATGTCGC CTACGGTTTT GCGGCTACCG CCTTCCCTGG TGGCAATGAG GCGTCGTGGT 360

GCTGTGCCTG CTATGCTCTT CAATTCACAT CCGGCCCCGT TGCTGGCAAG ACGATGGTTG 420

TGCAATCCAC CAACACTGGC GGAGATCTCA GCGGCACTCA CTTCGATATC CAGATGCCCG 480

GTGGAGGTCT CGGCATCTTC GACGGCTGCA CCCCGCAGTT CGGCTTCACG TTCCCCGGCA 540

ACCGCTACGG CGGTACCACG AGCCGCAGCC AGTGCGCCGA GCTGCCCTCC GTCCTCCGTG 600

ACGGCTGCCA CTGGCGTTAC GACTGGTTCA ACGATGCCGA CAACCCCAAC GTCAACTGGC 660

GCCGCGTCCG ATGCCCGGCG GCCCTCACGA ACCGCTCCGG CTGCGTCCGC AACGACGACA 720

ACAGCTACCC CGTCTTCGAG CCCGGCACGG GCACCCCGCC GACCCCCACG ACCACGACTA 780

CCAGCTCCCC TCCTCAGCCC ACCAACGGCG GAGGCGGCGG CACTTCTCCT CACTGGGGCC 840

AGTGCGGCGC CCAGGGCTGG TCTGGCCCGA CGGCCTGTGC CGGTGGGTCG ACCTGCAACC
900

TGATCAACCC GTGGTACTCC CAGTGCATTC CCAACTAAGT GATCCGGGCA TTGCGGTCGA
960

AAGGGGACCG TTAGTCGACA AGGCCCAGCC AGACCTCAGG CAGGTGGCTG CCATGGCAGA
1020

TTGTATATAG TCTTCCGAGT ACATACTATT GAATGAAAAT AAGAGCGGCT CGGACCATGA
1080

GCAGATGCCA TTTGATAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
1140

AAAAAAAAAA AAAA
1154

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 295 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Arg Ser Thr Ser Ile Leu Ile Gly Leu Val Ala Gly Val Ala Ala

1 5 10 15

Gln Ser Ser Gly Ser Gly His Thr Thr Arg Tyr Trp Asp Cys Cys Lys

20 25 30

Pro Ser Cys Ala Trp Asp Glu Lys Ala Ala Val Ser Arg Pro Val Thr

35 40 45

Thr Cys Asp Arg Asn Asn Ser Pro Leu Ser Pro Gly Ala Val Ser Gly

50 55 60

Cys Asp Pro Asn Gly Val Ala Phe Thr Cys Asn Asp Asn Gln Pro Trp

65 70 75 80

Ala Val Asn Asn Asn Val Ala Tyr Gly Phe Ala Ala Thr Ala Phe Pro

85 90 95

Gly Gly Asn Glu Ala Ser Trp Cys Cys Ala Cys Tyr Ala Leu Gln Phe

100 105 110

Thr Ser Gly Pro Val Ala Gly Lys Thr Met Val Val Gln Ser Thr Asn

115 120 125

Thr Gly Gly Asp Leu Ser Gly Thr His Phe Asp Ile Gln Met Pro Gly

130 135 140

Gly Gly Leu Gly Ile Phe Asp Gly Cys Thr Pro Gln Phe Gly Phe Thr

145 150 155 160

Phe Pro Gly Asn Arg Tyr Gly Gly Thr Thr Ser Arg Ser Gln Cys Ala

165 170 175

Glu Leu Pro Ser Val Leu Arg Asp Gly Cys His Trp Arg Tyr Asp Trp
180 185 190

Phe Asn Asp Ala Asp Asn Pro Asn Val Asn Trp Arg Arg Val Arg Cys
195 200 205

Pro Ala Ala Leu Thr Asn Arg Ser Gly Cys Val Arg Asn Asp Asp Asn
210 215 220

Ser Tyr Pro Val Phe Glu Pro Gly Thr Gly Thr Pro Pro Thr Pro Thr
225 230 235 240

Thr Thr Thr Thr Ser Ser Pro Pro Gln Pro Thr Asn Gly Gly Gly Gly
245 250 255

Gly Thr Ser Pro His Trp Gly Gln Cys Gly Gly Gln Gly Trp Ser Gly
260 265 270

Pro Thr Ala Cys Ala Gly Gly Ser Thr Cys Asn Leu Ile Asn Pro Trp
275 280 285

Tyr Ser Gln Cys Ile Pro Asn
290 295

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1423 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae, DSM 10080

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AAAGTTCTGG CCGGAACAGA TCTCCGTTGT CGATCTTCGA TTTTCCAGAC TCAGTCTGTG 60

ACACTCCTTC AATCCACATT CCTTTACTTC TTCGTCACTC ATTCACATCA TGATTTCAGC 120

TTGGATTCTC CTGGGGCTGG TAGGCGCCGT GCCCTCCTCC GTCATGGCCG CCTCGGGCAA 180

AGGCCACACC ACCCGCTACT GGGATTGCTG CAAGACTTCT TGCGCATGGG AGGGCAAGGC 240

ATCCGTCTCC GAGCCTGTCC TGACCTGTAA CAAGCAGGAC AACCCCATCG TCGATGCCAA 300

CGCCAGAAGC GGCTGCGACG GCGGCGGGGC ATTTGCCTGT ACCAACAATT CCCCTTGGGC 360

CGTGAGCGAG GACCTGGCCT ACGGATTTGC TGCCACAGCC CTCAGCGGCG GCACTGAGGG 420

CAGCTGGTGC TGCGCGTGTT ACGCCATCAC ATTCACGAGT GGCCCTGTGG CTGGCAAGAA 480

GATGGTCGTC CAGTCCACGA ACACGGGAGG CGACCTGTCC AACAACCACT TTGACCTGAT 540

GATTCCCGGT GGAGGCCTCG GCATCTTTGA CGGTTGCTCG GCTCAGTTCG GACAACTTCT 600

TCCCGGCGAG CGTTACGGAG GTGTTTCGTC CCGCTCTCAA TGCGATGGCA TGCCCGAGCT
660

CTTGAAAGAC GGTTGCCAGT GGCGCTTCGA CTGGTTCAAG AACTCAGACA ACCCTGACAT
720

CGAGTTCGAG CAGGTCCAGT GTCCCAAAGA GCTCATTGCG GTCTCTGGGT GCGTCCGTGA
780

CGACGATAGC AGCTTTCCCG TCTTCCAAGG TTCGGGCTCA GGAGATGTCA ACCCACCTCC
840

CAAGCCGACT ACGACTACGA CCTCGTCAAA GCCGAAAACA ACCTCTGCAC CATCCACTCT
900

CTCGAACCCA TCCGCCCCTC AACAGCCAGG GAACACTGAT AGACCTGCCG AGACAACCAC
960

TACCAAGCTG CCTGCCCTGC CGGCCACGAC GAGCAGCCCT GCTGTCTCAG TTCCTTCGTC
1020

CAGCGCTCGC GTGCCTTTGT GGGGGCAATG CGACTCGGAA GCTTCATGGG ACGCACCTAA
1080

GAAGTGTGCA AAGGGCACCA AGTGTGTCTA CGTCAACGAC TGGTACTCTC AATGCCAGCC
1140

GAAGAACTCT TGTGCTTGAG AAGCAATGCT CACAGCATGT CCTCTTGTCA CCCCTTCTTT
1200

TCATTCCCAA ACATACTTAC TGTATTATTA TTTCCGATGC TTCATTTCTT GCTTGTTTCT
1260

GTCTTTCCTG CACGCAGCTT TCAACGATAC CCTTCATGCG ATTGCCCTAC GATCAGATGA
1320

TGGGCACGAC ATGGAGGATG GTTTGGGCAC TCACGCGTTC AGGACGGGAA AATTTATTAG
1380

GGCTGAGATC CGTGAATTGA CTTCATTTCG GCGGAATGTC TGC
1423

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 349 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Ile Ser Ala Trp Ile Leu Leu Gly Leu Val Gly Ala Val Pro Ser
1               5                   10                  15

Ser Val Met Ala Ala Ser Gly Lys Gly His Thr Thr Arg Tyr Trp Asp
            20                  25                  30

Cys Cys Lys Thr Ser Cys Ala Trp Glu Gly Lys Ala Ser Val Ser Glu
        35                  40                  45

Pro Val Leu Thr Cys Asn Lys Gln Asp Asn Pro Ile Val Asp Ala Asn
    50                  55                  60

Ala Arg Ser Gly Cys Asp Gly Gly Gly Ala Phe Ala Cys Thr Asn Asn
65                  70                  75                  80

Ser Pro Trp Ala Val Ser Glu Asp Leu Ala Tyr Gly Phe Ala Ala Thr
                85                  90                  95
```

Ala Leu Ser Gly Gly Thr Glu Gly Ser Trp Cys Cys Ala Cys Tyr Ala
100 105 110

Ile Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Lys Met Val Val Gln
115 120 125

Ser Thr Asn Thr Gly Gly Asp Leu Ser Asn Asn His Phe Asp Leu Met
130 135 140

Ile Pro Gly Gly Gly Leu Gly Ile Phe Asp Gly Cys Ser Ala Gln Phe
145 150 155 160

Gly Gln Leu Leu Pro Gly Glu Arg Tyr Gly Gly Val Ser Ser Arg Ser
165 170 175

Gln Cys Asp Gly Met Pro Glu Leu Leu Lys Asp Gly Cys Gln Trp Arg
180 185 190

Phe Asp Trp Phe Lys Asn Ser Asp Asn Pro Asp Ile Glu Phe Glu Gln
195 200 205

Val Gln Cys Pro Lys Glu Leu Ile Ala Val Ser Gly Cys Val Arg Asp
210 215 220

Asp Asp Ser Ser Phe Pro Val Phe Gln Gly Ser Gly Ser Gly Asp Val
225 230 235 240

Asn Pro Pro Pro Lys Pro Thr Thr Thr Thr Thr Ser Ser Lys Pro Lys
245 250 255

Thr Thr Ser Ala Pro Ser Thr Leu Ser Asn Pro Ser Ala Pro Gln Gln
260 265 270

Pro Gly Asn Thr Asp Arg Pro Ala Glu Thr Thr Thr Thr Lys Leu Pro
275 280 285

Ala Leu Pro Ala Thr Thr Ser Ser Pro Ala Val Ser Val Pro Ser Ser
290 295 300

Ser Ala Arg Val Pro Leu Trp Gly Gln Cys Asp Ser Glu Ala Ser Trp
305 310 315 320

Asp Ala Pro Lys Lys Cys Ala Lys Gly Thr Lys Cys Val Tyr Val Asn
325 330 335

Asp Trp Tyr Ser Gln Cys Gln Pro Lys Asn Ser Cys Ala
340 345

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1174 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae, DSM 10081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

GAGCAGCACC CCTCAAGCTG TACAGTTTCC ACCCCGCTCT CTTTTCTTCG GCCCCCAGGA 60

TGCGCTCTAC TCCCGTTCTT CGCACAACCC TGGCCGCTGC ACTTCCTCTG GTCGCCTCCG 120

CGGCCAGTGG CAGTGGCCAG TCCACGAGAT ACTGGGACTG CTGCAAGCCG TCGTGCGCTT 180

GGCCCGGGAA GGCCGCCGTC AGCCAACCGG TCTACGCGTG CGATGCCAAC TTCCAGCGCC 240

TGTCCGACTT CAATGTCCAG TCGGGCTGCA ACGGCGGCTC GGCCTACTCC TGCGCCGACC 300

AGACTCCCTG GGCGGTGAAC GACAATCTCG CCTACGGCTT CGCCGCGACG AGCATCGCCG 360

GCGGGTCCGA ATCCTCGTGG TGCTGCGCCT GCTACGCGCT CACCTTCACT TCCGGTCCCG 420

TCGCCGGCAA GACAATGGTG GTGCAGTCAA CGAGCACTGG CGGCGACCTG GGAAGTAACC 480

AGTTCGATAT CGCCATGCCC GGCGGCGGCG TGGGCATCTT CAACGGCTGC AGCTCGCAGT 540

TCGGCGGCCT CCCCGGCGCT CAATACGGCG GCATTTCGTC GCGCGACCAG TGCGATTCCT 600

TCCCCGCGCC GCTCAAGCCC GGCTGCCAGT GGCGGTTTGA CTGGTTCCAG AACGCCGACA 660

ACCCGACGTT CACGTTCCAG CAGGTGCAGT GCCCCGCCGA GATCGTTGCC CGCTCCGGCT 720

GCAAGCGCAA CGACGACTCC AGCTTCCCCG TCTTCACCCC CCCAAGCGGT GGCAACGGTG 780

GCACCGGGAC GCCCACGTCG ACTGCGCCTG GGTCGGGCCA GACGTCTCCC GGCGGCGGCA 840

GTGGCTGCAC GTCTCAGAAG TGGGCTCAGT GCGGTGGCAT CGGCTTCAGC GGATGCACCA 900

CCTGTGTCTC TGGCACCACC TGCCAGAAGT TGAACGACTA CTACTCGCAG TGCCTCTAAA 960

CAGCTTTTCG CACGAGGTGG CGGGACGGAG CAAGGAGACC GTCAACTTCG TCATGCATAT 1020

TTTTTGAGCG CTCAATACAT ACATAACCTT CGATTCTTGT ACATAGCACG CCGGTACACA 1080

TCTCACACCG ACTTTGGGGG CGGAATCAGG CCCGTTTTAA AAAAAAAAAA AAAAAAAAAA 1140

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAA 1174

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 299 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Arg Ser Thr Pro Val Leu Arg Thr Thr Leu Ala Ala Ala Leu Pro
1 5 10 15

Leu Val Ala Ser Ala Ala Ser Gly Ser Gly Gln Ser Thr Arg Tyr Trp

20 25 30

Asp Cys Cys Lys Pro Ser Cys Ala Trp Pro Gly Lys Ala Ala Val Ser

35 40 45

Gln Pro Val Tyr Ala Cys Asp Ala Asn Phe Gln Arg Leu Ser Asp Phe

50 55 60

Asn Val Gln Ser Gly Cys Asn Gly Gly Ser Ala Tyr Ser Cys Ala Asp

65 70 75 80

Gln Thr Pro Trp Ala Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala Ala

85 90 95

Thr Ser Ile Ala Gly Gly Ser Glu Ser Ser Trp Cys Cys Ala Cys Tyr

100 105 110

Ala Leu Thr Phe Thr Ser Gly Pro Val Ala Gly Lys Thr Met Val Val

115 120 125

Gln Ser Thr Ser Thr Gly Gly Asp Leu Gly Ser Asn Gln Phe Asp Ile

130 135 140

Ala Met Pro Gly Gly Gly Val Gly Ile Phe Asn Gly Cys Ser Ser Gln

145 150 155 160

Phe Gly Gly Leu Pro Gly Ala Gln Tyr Gly Gly Ile Ser Ser Arg Asp
165 170 175

Gln Cys Asp Ser Phe Pro Ala Pro Leu Lys Pro Gly Cys Gln Trp Arg
180 185 190

Phe Asp Trp Phe Gln Asn Ala Asp Asn Pro Thr Phe Thr Phe Gln Gln
195 200 205

Val Gln Cys Pro Ala Glu Ile Val Ala Arg Ser Gly Cys Lys Arg Asn
210 215 220

Asp Asp Ser Ser Phe Pro Val Phe Thr Pro Pro Ser Gly Gly Asn Gly
225 230 235 240

Gly Thr Gly Thr Pro Thr Ser Thr Ala Pro Gly Ser Gly Gln Thr Ser
245 250 255

Pro Gly Gly Gly Ser Gly Cys Thr Ser Gln Lys Trp Ala Gln Cys Gly
260 265 270

Gly Ile Gly Phe Ser Gly Cys Thr Thr Cys Val Ser Gly Thr Thr Cys
275 280 285

Gln Lys Leu Asn Asp Tyr Tyr Ser Gln Cys Leu
290 295

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 913 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli, DSM 10512

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCACTATTCT CAGCTCCATT CTCCCTTGAA GTAATTCACC ATGTTCTCTC CGCTCTGGGC
60

CCTGTCGGCT CTGCTCCTAT TTCCTGCCAC TGAAGCCACT AGCGGCGTGA CAACCAGGTA
120

CTGGGACTGC TGCAAGCCGT CTTGTGCTTG GACGGGCAAA GCATCCGTCT CCAAGCCCGT
180

CGGAACCTGC GACATCAACG ACAACGCCCA GACGCCGAGC GATCTGCTCA AGTCGTCCTG
240

TGATGGCGGC AGCGCCTACT ACTGCAGCAA CCAGGGCCCA TGGGCCGTGA ACGACAGCCT
300

TTCCTACGGC TTCGCTGCCG CCAAGCTGTC CGGAAAGCAG GAGACTGATT GGTGCTGTGG
360

CTGCTACAAG CTCACATTCA CCTCCACCGC CGTTTCCGGC AAGCAAATGA TCGTGCAAAT
420

CACGAACACG GGCGGCGACC TCGGCAACAA CCACTTCGAC ATCGCCATGC CGGGCGGCGG
480

CGTCGGCATC TTCAACGGGT GCTCCAAGCA ATGGAACGGC ATCAATCTGG GCAACCAGTA
540

TGGCGGCTTC ACTGACCGCT CGCAATGTGC GACGCTCCCG TCCAAGTGGC AGGCCAGCTG 600

CAACTGGCGC TTCGACTGGT TCGAGAATGC CGACAACCCC ACCGTCGATT GGGAGCCTGT 660

CACTTGCCCA CAGGAATTGG TCGCCCCGGAC TGGCTGTTCC CGTACCTAAG TGGGGGTGGA 720

ACCTCCATGT GAATTGGTGT ATATAGCTCC TGCCTGAGCA TCCACCAGTT CGCATGTGTT 780

GATCAGGAGT TGTGTTGCCT TGCTAGGAAA GACTTTGTTG GAAACTTGCG TGTTTATTCC 840

AATTGAATAA CCCTGTATAG ACCGGTCACA TTTTTCTCTG AAAAAAAAAA AAAAAAAAAA 900

AAAAAAAAAA AAA 913

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 222 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Phe Ser Pro Leu Trp Ala Leu Ser Ala Leu Leu Leu Phe Pro Ala
1 5 10 15

Thr Glu Ala Thr Ser Gly Val Thr Thr Arg Tyr Trp Asp Cys Cys Lys
20 25 30

Pro Ser Cys Ala Trp Thr Gly Lys Ala Ser Val Ser Lys Pro Val Gly

35 40 45

Thr Cys Asp Ile Asn Asp Asn Ala Gln Thr Pro Ser Asp Leu Leu Lys

50 55 60

Ser Ser Cys Asp Gly Gly Ser Ala Tyr Tyr Cys Ser Asn Gln Gly Pro

65 70 75 80

Trp Ala Val Asn Asp Ser Leu Ser Tyr Gly Phe Ala Ala Ala Lys Leu

85 90 95

Ser Gly Lys Gln Glu Thr Asp Trp Cys Cys Gly Cys Tyr Lys Leu Thr

100 105 110

Phe Thr Ser Thr Ala Val Ser Gly Lys Gln Met Ile Val Gln Ile Thr

115 120 125

Asn Thr Gly Gly Asp Leu Gly Asn Asn His Phe Asp Ile Ala Met Pro

130 135 140

Gly Gly Gly Val Gly Ile Phe Asn Gly Cys Ser Lys Gln Trp Asn Gly

145 150 155 160

Ile Asn Leu Gly Asn Gln Tyr Gly Gly Phe Thr Asp Arg Ser Gln Cys

165 170 175

Ala Thr Leu Pro Ser Lys Trp Gln Ala Ser Cys Asn Trp Arg Phe Asp
180 185 190

Trp Phe Glu Asn Ala Asp Asn Pro Thr Val Asp Trp Glu Pro Val Thr
195 200 205

Cys Pro Gln Glu Leu Val Ala Arg Thr Gly Cys Ser Arg Thr
210 215 220

2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 808 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli, DSM 10511

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCGCTGCTGG GTATATAATG CTCAGACTTG GAACCAATGG TCCATCCAAA CATGCTTAAA 60

ACGCTCGCTC CATTGATCAT CTTGGCCGCC TCGGTCACAG CGCAAACAGC AGGAGTTACG 120

ACCCGCTACT GGGACTGCTG CAAGCCAAGC TGTGGATGGA GTGGAAAGGC TTCTGTTTCT 180

GCTCCAGTCA GAACTTGCGA TCGTAATGGA AATACACTTG GCCCAGACGT GAAAAGCGGA 240

TGTGATAGCG GTGGAACGTC ATTCACTTGC GCGAACAATG GTCCATTTGC GATTGACAAT
300

AACACTGCAT ATGGTTTTGC TGCAGCCCAC TTAGCGGGCT CTAGCGAAGC AGCCTGGTGT
360

TGCCAGTGCT ACGAATTGAC GTTTACGAGT GGACCCGTAG TTGGGAAGAA ACTGACCGTT
420

CAAGTCACAA ACACGGGAGG TGACCTCGGA AATAATCACT TTGACCTGAT GATCCCCGGT
480

GGAGGTGTTG GCCTCTTCAC ACAAGGATGT CCTGCTCAGT TTGGGAGCTG GAACGGGGGT
540

GCTCAATACG GGGGTGTGTC CAGCCGTGAC CAATGCTCCC AACTTCCAGC AGCTGTGCAA
600

GCTGGATGTC AATTCCGTTT CGACTGGATG GGTGGCGCGG ATAACCCCAA CGTCACCTTC
660

CGACCTGTGA CCTGCCCAGC GCAGCTCACT AATATCTCGG GCTGTGTTCG TAAATGATTC
720

ACGAATATGT AGTGTCGAAT ATGTACATGT GTATGTACTA TAGCTTCAAA GATGGAGGGT
780

CTGTTTAAAA AAAAAAAAAA AAAAAAAA
808

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 226 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Val His Pro Asn Met Leu Lys Thr Leu Ala Pro Leu Ile Ile Leu
1 5 10 15

Ala Ala Ser Val Thr Ala Gln Thr Ala Gly Val Thr Thr Arg Tyr Trp
20 25 30

Asp Cys Cys Lys Pro Ser Cys Gly Trp Ser Gly Lys Ala Ser Val Ser
35 40 45

Ala Pro Val Arg Thr Cys Asp Arg Asn Gly Asn Thr Leu Gly Pro Asp
50 55 60

Val Lys Ser Gly Cys Asp Ser Gly Gly Thr Ser Phe Thr Cys Ala Asn
65 70 75 80

Asn Gly Pro Phe Ala Ile Asp Asn Asn Thr Ala Tyr Gly Phe Ala Ala
85 90 95

Ala His Leu Ala Gly Ser Ser Glu Ala Ala Trp Cys Cys Gln Cys Tyr
100 105 110

Glu Leu Thr Phe Thr Ser Gly Pro Val Val Gly Lys Lys Leu Thr Val
115 120 125

Gln Val Thr Asn Thr Gly Gly Asp Leu Gly Asn Asn His Phe Asp Leu

```
        130                 135                 140

Met Ile Pro Gly Gly Gly Val Gly Leu Phe Thr Gln Gly Cys Pro Ala
145                 150                 155                 160

Gln Phe Gly Ser Trp Asn Gly Gly Ala Gln Tyr Gly Gly Val Ser Ser
                165                 170                 175

Arg Asp Gln Cys Ser Gln Leu Pro Ala Ala Val Gln Ala Gly Cys Gln
            180                 185                 190

Phe Arg Phe Asp Trp Met Gly Gly Ala Asp Asn Pro Asn Val Thr Phe
        195                 200                 205

Arg Pro Val Thr Cys Pro Ala Gln Leu Thr Asn Ile Ser Gly Cys Val
    210                 215                 220

Arg Lys
225
```

(2) INFORMATION FOR SEQ ID NO: 14-A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1048 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14-A:

GACTTGGAAC CAATGGTCCA TCCAAACATG CTTAAAACGC TCGCTCCATT GATCATCTTG 60

GCCGCCTCGG TCACAGCGCA AACAGCAGGA GTTACGACCC GCTACTGGGA CTGCTGCAAG 120

CCAAGCTGTG GATGGAGTGG AAAGGCTTCT GTTTCTGCTC CAGTCAGAAC TTGCGATCGT 180

AATGGAAATA CACTTGGCCC AGACGTGAAA AGCGGATGTG ATAGCGGTGG AACGTCATTC 240

ACTTGCGCGA ACAATGGTCC ATTTGCGATT GACAATAACA CTGCATATGG TTTTGCTGCA 300

GCCCACTTAG CGGGCTCTAG CGAAGCAGCC TGGTGTTGCC AGTGCTACGA ATTGACGTTT 360

ACGAGTGGAC CCGTAGTTGG GAAGAAACTG ACCGTTCAAG TCACAAACAC GGGAGGTGAC 420

CTCGGAAATA ATCACTTTGA CCTGATGATC CCCGGTGGAG GTGTTGGCCT CTTCACACAA 480

GGATGTCCTG CTCAGTTTGG GAGCTGGAAC GGGGGTGCTC AATACGGGGG TGTGTCCAGC 540

CGTGACCAAT GCTCCCAACT TCCAGCAGCT GTGCAAGCTG GATGTCAATT CCGTTTCGAC 600

TGGATGGGTG GCGCGGATAA CCCCAACGTC ACCTTCCGAC CTGTGACCTG CCCAGCGCAG 660

CTCACTAATA TCTCGGGCTG TGTTCGTAAA CCCTCCAGCA GCACCAGCTC TCCGGTCAAC 720

CAGCCTACCA GCACCAGCAC CACGTCCACC TCCACCACCT CGAGCCCGCC AGTCCAGCCT 780

ACGACTCCCA GCGGCTGCAC TGCTGAGAGG TGGGCTCAGT GCGGCGGCAA TGGCTGGAGC 840

GGCTGCACCA CCTGCGTCGC TGGCAGCACT TGCACGAAGA TTAATGACTG GTACCATCAG
900

TGCCTGTAGA CGCAGGGCAG CTTGAGGGCC TTACTGGTGG CGCAACGAAA TGACACTCCC
960

AATCACTGTA TTAGTTCTTG TACATAATTT CGTCATCCCT CCAGGGATTG TCACATAAAT
1020

GCAATGAGGA ACAATGAGTA CAGAATTC
1048

(2) INFORMATION FOR SEQ ID NO: 14-B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 298 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14-B:

Met Val His Pro Asn Met Leu Lys Thr Leu Ala Pro Leu Ile Ile Leu
1 5 10 15

Ala Ala Ser Val Thr Ala Gln Thr Ala Gly Val Thr Thr Arg Tyr Trp
20 25 30

Asp Cys Cys Lys Pro Ser Cys Gly Trp Ser Gly Lys Ala Ser Val Ser
35 40 45

Ala Pro Val Arg Thr Cys Asp Arg Asn Gly Asn Thr Leu Gly Pro Asp
50 55 60

Val Lys Ser Gly Cys Asp Ser Gly Gly Thr Ser Phe Thr Cys Ala Asn
65 70 75 80

Asn Gly Pro Phe Ala Ile Asp Asn Asn Thr Ala Tyr Gly Phe Ala Ala
85 90 95

Ala His Leu Ala Gly Ser Ser Glu Ala Ala Trp Cys Cys Gln Cys Tyr
100 105 110

Glu Leu Thr Phe Thr Ser Gly Pro Val Val Gly Lys Lys Leu Thr Val
115 120 125

Gln Val Thr Asn Thr Gly Gly Asp Leu Gly Asn Asn His Phe Asp Leu
130 135 140

Met Ile Pro Gly Gly Gly Val Gly Leu Phe Thr Gln Gly Cys Pro Ala
145 150 155 160

Gln Phe Gly Ser Trp Asn Gly Gly Ala Gln Tyr Gly Gly Val Ser Ser
165 170 175

Arg Asp Gln Cys Ser Gln Leu Pro Ala Ala Val Gln Ala Gly Cys Gln
180 185 190

Phe Arg Phe Asp Trp Met Gly Gly Ala Asp Asn Pro Asn Val Thr Phe

195 200 205

Arg Pro Val Thr Cys Pro Ala Gln Leu Thr Asn Ile Ser Gly Cys Val

210 215 220

Arg Lys Pro Ser Ser Ser Thr Ser Ser Pro Val Asn Gln Pro Thr Ser

225 230 235 240

Thr Ser Thr Thr Ser Thr Ser Thr Thr Ser Ser Pro Pro Val Gln Pro

245 250 255

Thr Thr Pro Ser Gly Cys Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly

260 265 270

Asn Gly Trp Ser Gly Cys Thr Thr Cys Val Ala Gly Ser Thr Cys Thr

275 280 285

Lys Ile Asn Asp Trp Tyr His Gln Cys Leu

290 295

(2) INFORMATION FOR SEQ ID NO: 15-A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1031 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15-A:

CCATCCAAAC ATGCTTAAAA CGCTCGCTCC ATTGATCATC TTGGCCGCCT CGGTCACAGC 60

GCAAACAGCA GGAGTTACGA CCCGCTACTG GGACTGCTGC AAGCCAAGCT GTGGATGGAG 120

TGGAAAGGCT TCTGTTTCTG CTCCAGTCAG AACTTGCGAT CGTAATGGAA ATACACTTGG 180

CCCAGACGTG AAAAGCGGAT GTGATAGCGG TGGAACGTCA TTCACTTGCG CGAACAATGG 240

TCCATTTGCG ATTGACAATA ACACTGCATA TGGTTTTGCT GCAGCCCACT TAGCGGGCTC 300

TAGCGAAGCA GCCTGGTGTT GCCAGTGCTA CGAATTGACG TTTACGAGTG GACCCGTAGT 360

TGGGAAGAAA CTGACCGTTC AAGTCACAAA CACGGGAGGT GACCTCGGAA ATAATCACTT 420

TGACCTGATG ATCCCCGGTG GAGGTGTTGG CCTCTTCACA CAAGGATGTC CTGCTCAGTT 480

TGGGAGCTGG AACGGGGGTG CTCAATACGG GGGTGTGTCC AGCCGTGACC AATGCTCCCA 540

ACTTCCAGCA GCTGTGCAAG CTGGATGTCA ATTCCGTTTC GACTGGATGG GTGGCGCGGA 600

TAACCCCAAC GTCACCTTCC GACCTGTGAC CTGCCCAGCG CAGCTCACTA ATATCTCGGG 660

CTGTGTTCGT AAACCCTCCA GCAGCACCAG CTCTCCGGTC AACCAGCCTA CCAGCACCAG 720

CACCACGTCC ACCTCCACCA CCTCGAGCCC GCCAGTCCAG CCTACGACTC CCAGCGGCTG 780

CACTGCTGAG AGGTGGGCTC AGTGCGGCGG CAATGGCTGG AGCGGCTGCA CCACCTGCGT 840

CGCTGGCAGC ACTTGCACGA AGATTAATGA CTGGTACCAT CAGTGCCTGT AGACGCAGGG
900

CAGCTTGAGG GCCTTACTGG TGGCGCAACG AAATGACACT CCCAATCACT GTATTAGTTC
960

TTGTACATAA TTTCGTCATC CCTCCAGGGA TTGTCACATA AATGCAATGA GGAACAATGA
1020

GTACAGAATT C
1031

(2) INFORMATION FOR SEQ ID NO: 15-B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15-B:

Met Leu Lys Thr Leu Ala Pro Leu Ile Ile Leu Ala Ala Ser Val Thr
1 5 10 15

Ala Gln Thr Ala Gly Val Thr Thr Arg Tyr Trp Asp Cys Cys Lys Pro
20 25 30

Ser Cys Gly Trp Ser Gly Lys Ala Ser Val Ser Ala Pro Val Arg Thr
35 40 45

Cys Asp Arg Asn Gly Asn Thr Leu Gly Pro Asp Val Lys Ser Gly Cys
50 55 60

Asp Ser Gly Gly Thr Ser Phe Thr Cys Ala Asn Asn Gly Pro Phe Ala
65 70 75 80

Ile Asp Asn Asn Thr Ala Tyr Gly Phe Ala Ala Ala His Leu Ala Gly
85 90 95

Ser Ser Glu Ala Ala Trp Cys Cys Gln Cys Tyr Glu Leu Thr Phe Thr
100 105 110

Ser Gly Pro Val Val Gly Lys Lys Leu Thr Val Gln Val Thr Asn Thr
115 120 125

Gly Gly Asp Leu Gly Asn Asn His Phe Asp Leu Met Ile Pro Gly Gly
130 135 140

Gly Val Gly Leu Phe Thr Gln Gly Cys Pro Ala Gln Phe Gly Ser Trp
145 150 155 160

Asn Gly Gly Ala Gln Tyr Gly Gly Val Ser Ser Arg Asp Gln Cys Ser
165 170 175

Gln Leu Pro Ala Ala Val Gln Ala Gly Cys Gln Phe Arg Phe Asp Trp
180 185 190

Met Gly Gly Ala Asp Asn Pro Asn Val Thr Phe Arg Pro Val Thr Cys

195 200 205

Pro Ala Gln Leu Thr Asn Ile Ser Gly Cys Val Arg Lys Pro Ser Ser
210 215 220

Ser Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr Ser
225 230 235 240

Thr Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser Gly
245 250 255

Cys Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser Gly
260 265 270

Cys Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp Trp
275 280 285

Tyr His Gln Cys Leu
290

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli, DSM 10571

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

CAACAGTTCA AACACCTACA AGGTCCCGTG CCCTGTAGAC CATGCGTTCC TCTGCAGTCC 60

TCATCGGCCT CGTGGCCGGT GTGGCCGCCC AGTCCTCTGG CACCGGCCGC ACCACCAGAT 120

ACTGGGACTG CTGCAAGCCG TCCTGCGGGT GGGACGAAAA GGCCTCCGTC AGCCAGCCCG 180

TCAAGACGTG CGATAGGAAC AACAACCCTC TCGCGTCCAC GGCCAGGAGC GGCTGCGATT 240

CCAACGGCGT CGCCTACACG TGCAACGATA ACCAGCCGTG GGCTGTCAAC GATAACCTGG 300

CCTATGGTTT TGCTGCCACG GCTTTCAGTG GTGGATCGGA GGCCAGCTGG TGCTGTGCCT 360

GCTATGCCCT TCAGTTCACC TCCGGCCCTG TTGCGGGAAA GACCATGGTC GTCCAGTCGA 420

CAAACACCGG CGGCGACCTC AGCGGCAACC ACTTTGACAT CCTCATGCCC GGCGGCGGCC 480

TGGGCATCTT CGACGGCTGC ACCCCGCAAT GGGGCGTCAG CTTCCCCGGA AACCGCTACG 540

GCGGCACCAC CAGCCGCAGC CAGTGCTCCC AAATCCCCTC GGCCCTGCAG CCCGGCTGCA 600

ACTGGCGGTA CGACTGGTTC AACGACGCCG ACAACCCCGA CGTCTCGTGG CGCCGCGTCC 660

AGTGCCCCGC CGCACTCACC GACCGCACCG GCTGCCGCCG CTCCGATGAC GGGAACTATC 720

CCGTCTTCCA GCCCGGTCCG CCCCCGGCCA CGACGATCAG GACATCGACT ACCATCACAG 780

CCTCATCGTC GTCTTCGTCT TCGTCGTCGT CGACTACGGC TGGTAGCCCG CCTGTGCCGA
840

CTGGTGGTGG TAGTGGGCCA ACGTCGCCTG TCTGGGGACA GTGCGGCGGT CAGGGATGGA
900

GTGGTCCTAC GCGTTGTGTT GCTGGGTCGA CATGCAGTGT GGTCAACCCG TGGTACTCGC
960

AGTGTTTTCC TTAAGGAGCC TCTGGCTGAG CAGATCCTTT CGAAGAGGAG GGTCTCTCTG
1020

CTCTTTCAGT CTGTTCAGGG AACGGCCGTC TCGGCTACAT TGTACATATC CCACCTCGTA
1080

TATAGCTAGC TCATCTACAC TTGTGATCTC CAAAAAAAAA AAAAAAAAAA AA
1132

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 310 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Met Arg Ser Ser Ala Val Leu Ile Gly Leu Val Ala Gly Val Ala Ala

1 5 10 15

Gln Ser Ser Gly Thr Gly Arg Thr Thr Arg Tyr Trp Asp Cys Cys Lys

20 25 30

Pro Ser Cys Gly Trp Asp Glu Lys Ala Ser Val Ser Gln Pro Val Lys

35 40 45

Thr Cys Asp Arg Asn Asn Asn Pro Leu Ala Ser Thr Ala Arg Ser Gly

50 55 60

Cys Asp Ser Asn Gly Val Ala Tyr Thr Cys Asn Asp Asn Gln Pro Trp

65 70 75 80

Ala Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala Ala Thr Ala Phe Ser

85 90 95

Gly Gly Ser Glu Ala Ser Trp Cys Cys Ala Cys Tyr Ala Leu Gln Phe

100 105 110

Thr Ser Gly Pro Val Ala Gly Lys Thr Met Val Val Gln Ser Thr Asn

115 120 125

Thr Gly Gly Asp Leu Ser Gly Asn His Phe Asp Ile Leu Met Pro Gly

130 135 140

Gly Gly Leu Gly Ile Phe Asp Gly Cys Thr Pro Gln Trp Gly Val Ser

145 150 155 160

Phe Pro Gly Asn Arg Tyr Gly Gly Thr Thr Ser Arg Ser Gln Cys Ser

165 170 175

Gln Ile Pro Ser Ala Leu Gln Pro Gly Cys Asn Trp Arg Tyr Asp Trp

180 185 190

Phe Asn Asp Ala Asp Asn Pro Asp Val Ser Trp Arg Arg Val Gln Cys

195 200 205

Pro Ala Ala Leu Thr Asp Arg Thr Gly Cys Arg Arg Ser Asp Asp Gly

210 215 220

Asn Tyr Pro Val Phe Gln Pro Gly Pro Pro Pro Ala Thr Thr Ile Arg

225 230 235 240

Thr Ser Thr Thr Ile Thr Ala Ser Ser Ser Ser Ser Ser Ser Ser Ser

245 250 255

Ser Thr Thr Ala Gly Ser Pro Pro Val Pro Thr Gly Gly Gly Ser Gly

260 265 270

Pro Thr Ser Pro Val Trp Gly Gln Cys Gly Gly Gln Gly Trp Ser Gly

275 280 285

Pro Thr Arg Cys Val Ala Gly Ser Thr Cys Ser Val Val Asn Pro Trp

290 295 300

Tyr Ser Gln Cys Phe Pro
305 310

(2) INFORMATION FOR SEQ ID NO: 18-A:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 885 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
"Construction from Macrophomina"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..885

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18-A:

ATG TTC TCT CCG CTC TGG GCC CTG TCG GCT CTG CTC CTA TTT CCT GCC
48
Met Phe Ser Pro Leu Trp Ala Leu Ser Ala Leu Leu Leu Phe Pro Ala
145 150 155

ACT GAA GCC ACT AGC GGC GTG ACA ACC AGG TAC TGG GAC TGC TGC AAG
96
Thr Glu Ala Thr Ser Gly Val Thr Thr Arg Tyr Trp Asp Cys Cys Lys
160 165 170

CCG TCT TGT GCT TGG ACG GGC AAA GCA TCC GTC TCC AAG CCC GTC GGA
144
Pro Ser Cys Ala Trp Thr Gly Lys Ala Ser Val Ser Lys Pro Val Gly
175 180 185

ACC TGC GAC ATC AAC GAC AAC GCC CAG ACG CCG AGC GAT CTG CTC AAG
192
Thr Cys Asp Ile Asn Asp Asn Ala Gln Thr Pro Ser Asp Leu Leu Lys
190 195 200 205

TCG TCC TGT GAT GGC GGC AGC GCC TAC TAC TGC AGC AAC CAG GGC CCA

240
Ser Ser Cys Asp Gly Gly Ser Ala Tyr Tyr Cys Ser Asn Gln Gly Pro
210 215 220

TGG GCC GTG AAC GAC AGC CTT TCC TAC GGC TTC GCT GCC GCC AAG CTG
288
Trp Ala Val Asn Asp Ser Leu Ser Tyr Gly Phe Ala Ala Ala Lys Leu
225 230 235

TCC GGA AAG CAG GAG ACT GAT TGG TGC TGT GGC TGC TAC AAG CTC ACA
336
Ser Gly Lys Gln Glu Thr Asp Trp Cys Cys Gly Cys Tyr Lys Leu Thr
240 245 250

TTC ACC TCC ACC GCC GTT TCC GGC AAG CAA ATG ATC GTG CAA ATC ACG
384
Phe Thr Ser Thr Ala Val Ser Gly Lys Gln Met Ile Val Gln Ile Thr
255 260 265

AAC ACG GGC GGC GAC CTC GGC AAC AAC CAC TTC GAC ATC GCC ATG CCG
432
Asn Thr Gly Gly Asp Leu Gly Asn Asn His Phe Asp Ile Ala Met Pro
270 275 280 285

GGC GGC GGC GTC GGC ATC TTC AAC GGG TGC TCC AAG CAA TGG AAC GGC
480
Gly Gly Gly Val Gly Ile Phe Asn Gly Cys Ser Lys Gln Trp Asn Gly
290 295 300

ATC AAT CTG GGC AAC CAG TAT GGC GGC TTC ACT GAC CGC TCG CAA TGT
528
Ile Asn Leu Gly Asn Gln Tyr Gly Gly Phe Thr Asp Arg Ser Gln Cys
305 310 315

GCG ACG CTC CCG TCC AAG TGG CAG GCC AGC TGC AAC TGG CGC TTC GAC
576
Ala Thr Leu Pro Ser Lys Trp Gln Ala Ser Cys Asn Trp Arg Phe Asp
320 325 330

TGG TTC GAG AAT GCC GAC AAC CCC ACC GTC GAT TGG GAG CCT GTC ACT
624
Trp Phe Glu Asn Ala Asp Asn Pro Thr Val Asp Trp Glu Pro Val Thr
335 340 345

TGC CCA CAG GAA TTG GTC GCC CGG ACT GGC TGT TCC CGT ACC CCC TCC
672
Cys Pro Gln Glu Leu Val Ala Arg Thr Gly Cys Ser Arg Thr Pro Ser
350 355 360 365

AGC AGC ACC AGC TCT CCG GTC AAC CAG CCT ACC AGC ACC AGC ACC ACG
720
Ser Ser Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr
370 375 380

TCC ACC TCC ACC ACC TCG AGC CCG CCA GTC CAG CCT ACG ACT CCC AGC
768
Ser Thr Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser
385 390 395

GGC TGC ACT GCT GAG AGG TGG GCT CAG TGC GGC GGC AAT GGC TGG AGC
816
Gly Cys Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser
400 405 410

GGC TGC ACC ACC TGC GTC GCT GGC AGC ACT TGC ACG AAG ATT AAT GAC
864
Gly Cys Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp
415 420 425

TGG TAC CAT CAG TGC CTG TAG
885
Trp Tyr His Gln Cys Leu *
430 435

(2) INFORMATION FOR SEQ ID NO: 18-B:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 295 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18-B:

Met Phe Ser Pro Leu Trp Ala Leu Ser Ala Leu Leu Leu Phe Pro Ala

```
1               5                   10                  15
Thr Glu Ala Thr Ser Gly Val Thr Thr Arg Tyr Trp Asp Cys Cys Lys
            20                  25                  30
Pro Ser Cys Ala Trp Thr Gly Lys Ala Ser Val Ser Lys Pro Val Gly
        35                  40                  45
Thr Cys Asp Ile Asn Asp Asn Ala Gln Thr Pro Ser Asp Leu Leu Lys
    50                  55                  60
Ser Ser Cys Asp Gly Gly Ser Ala Tyr Tyr Cys Ser Asn Gln Gly Pro
65                  70                  75                  80
Trp Ala Val Asn Asp Ser Leu Ser Tyr Gly Phe Ala Ala Ala Lys Leu
                85                  90                  95
Ser Gly Lys Gln Glu Thr Asp Trp Cys Cys Gly Cys Tyr Lys Leu Thr
            100                 105                 110
Phe Thr Ser Thr Ala Val Ser Gly Lys Gln Met Ile Val Gln Ile Thr
        115                 120                 125
Asn Thr Gly Gly Asp Leu Gly Asn Asn His Phe Asp Ile Ala Met Pro
    130                 135                 140
Gly Gly Gly Val Gly Ile Phe Asn Gly Cys Ser Lys Gln Trp Asn Gly
145                 150                 155                 160
Ile Asn Leu Gly Asn Gln Tyr Gly Gly Phe Thr Asp Arg Ser Gln Cys
                165                 170                 175
Ala Thr Leu Pro Ser Lys Trp Gln Ala Ser Cys Asn Trp Arg Phe Asp
            180                 185                 190
Trp Phe Glu Asn Ala Asp Asn Pro Thr Val Asp Trp Glu Pro Val Thr
        195                 200                 205
Cys Pro Gln Glu Leu Val Ala Arg Thr Gly Cys Ser Arg Thr Pro Ser
    210                 215                 220
Ser Ser Thr Ser Ser Pro Val Asn Gln Pro Thr Ser Thr Ser Thr Thr
225                 230                 235                 240
```

Ser Thr Ser Thr Thr Ser Ser Pro Pro Val Gln Pro Thr Thr Pro Ser
245 250 255

Gly Cys Thr Ala Glu Arg Trp Ala Gln Cys Gly Gly Asn Gly Trp Ser
260 265 270

Gly Cys Thr Thr Cys Val Ala Gly Ser Thr Cys Thr Lys Ile Asn Asp
275 280 285

Trp Tyr His Gln Cys Leu *
290 295

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli, DSM 10576

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..425

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CAAGATACAA TATGCGTTCC TCCACTATTT TGCAAACCGG CCTGGTGGCC GTTCTCCCCT
60

TCGCCGTCCA GGCCGCCTCA GGATCCGGCA AGTCCACCAG ATATTGGGAC TGCTGCAAAC
120

CATCTTGTGC CTGGTCCGGC AAGGCTTCTG TCAACCGCCC TGTTCTCGCC TGCAACGCAA
180

ACAACAACCC GCTGAACGAC GCCAACGTCA AGTCAGGATG TGATGGCGGT TCTGCATACA
240

CCTGTGCCAA CAACTCTCCC TGGGCAGTGA ATGACAATCT GGCCTACGGC TTCGCGGCCA
300

CAAAACTCAG CGGGGGGACC GAGTCATCTT GGTGCTGCGC CTGTTATGCC CTCACATTCA
360

CATCGGGTCC TGTTTCTGGC AAAACCTTGG TTGTCCAGTC TACCAGTACC GGTGGTGATC
420

TTGGC
425

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 141 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Met Arg Ser Ser Thr Ile Leu Gln Thr Gly Leu Val Ala Val Leu Pro
1 5 10 15

Phe Ala Val Gln Ala Ala Ser Gly Ser Gly Lys Ser Thr Arg Tyr Trp
20 25 30

Asp Cys Cys Lys Pro Ser Cys Ala Trp Ser Gly Lys Ala Ser Val Asn
35 40 45

Arg Pro Val Leu Ala Cys Asn Ala Asn Asn Asn Pro Leu Asn Asp Ala

50 55 60

Asn Val Lys Ser Gly Cys Asp Gly Gly Ser Ala Tyr Thr Cys Ala Asn
65 70 75 80

Asn Ser Pro Trp Ala Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala Ala
85 90 95

Thr Lys Leu Ser Gly Gly Thr Glu Ser Ser Trp Cys Cys Ala Cys Tyr
100 105 110

Ala Leu Thr Phe Thr Ser Gly Pro Val Ser Gly Lys Thr Leu Val Val
115 120 125

Gln Ser Thr Ser Thr Gly Gly Asp Leu Gly
130 135

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccobolus dilutellus
(B) STRAIN: CBS 275.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
TCG GCT TGC GAT AAC GGT GGT GGC ACT GCA TAC ATG TGT GCC AGC CAG

Ser Ala Cys Asp Asn Gly Gly Gly Thr Ala Tyr Met Cys Ala Ser Gln
  1               5                  10                  15

GAG CCG TGG GCA GTG AGC TCC AAC GTC GCG TAC GGC TTT GCT GCA GTT

Glu Pro Trp Ala Val Ser Ser Asn Val Ala Tyr Gly Phe Ala Ala Val
             20                  25                  30

AGA ATC AGC GGA

Arg Ile Ser Gly
         35
```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
Ser Ala Cys Asp Asn Gly Gly Gly Thr Ala Tyr Met Cys Ala Ser Gln
  1               5                  10                  15

Glu Pro Trp Ala Val Ser Ser Asn Val Ala Tyr Gly Phe Ala Ala Val
             20                  25                  30

Arg Ile Ser Gly
         35
```

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Thermomyces verrucosus
(B) STRAIN: CBS 285.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..99

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
GCC TGC AAC GCA AAC TTC CAG CGC ATC AGT GAC CCC AAC GCC AAG TCG
Ala Cys Asn Ala Asn Phe Gln Arg Ile Ser Asp Pro Asn Ala Lys Ser

GGC TGC GAT GGT GGC TCG GCC TTC TCT TGC GCC AAA CAA ACC CCT TGG
Gly Cys Asp Gly Gly Ser Ala Phe Ser Cys Ala Lys Gln Thr Pro Trp

GCC
Ala
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Ala Cys Asn Ala Asn Phe Gln Arg Ile Ser Asp Pro Asn Ala Lys Ser
1   5   10   15

Gly Cys Asp Gly Gly Ser Ala Phe Ser Cys Ala Lys Gln Thr Pro Trp
            20   25   30

Ala

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 225 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Xylaria hypoxylon
(B) STRAIN: CBS 284.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..225

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GAC CAG CCG CTC GGC GGA CAA CGG ACG CGA CCA AGG AGC GCG TGC GAC
48
Asp Gln Pro Leu Gly Gly Gln Arg Thr Arg Pro Arg Ser Ala Cys Asp
    35   40   45

AAT GGC GGC TCT GCA TAC ATG TGC AGC AAC CAG AGC CCG TGG GCC GTC
96
Asn Gly Gly Ser Ala Tyr Met Cys Ser Asn Gln Ser Pro Trp Ala Val
50 55 60 65

GAC GAT TCT CTC AGT TAC GGA TGG GCT GCC GTT AGG ATC TAT GGA CAT
144
Asp Asp Ser Leu Ser Tyr Gly Trp Ala Ala Val Arg Ile Tyr Gly His
70 75 80

ACC GAA ACT ACT TGG TGC TGC GCT TGC TAC GAG TTG ACT TTT ACC AGC
192
Thr Glu Thr Thr Trp Cys Cys Ala Cys Tyr Glu Leu Thr Phe Thr Ser
85 90 95

GGT CCG GTT AGC GGC AAG AAG ATG ATT GTT CAG
225
Gly Pro Val Ser Gly Lys Lys Met Ile Val Gln
100 105

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Asp Gln Pro Leu Gly Gly Gln Arg Thr Arg Pro Arg Ser Ala Cys Asp
1 5 10 15

Asn Gly Gly Ser Ala Tyr Met Cys Ser Asn Gln Ser Pro Trp Ala Val
20 25 30

Asp Asp Ser Leu Ser Tyr Gly Trp Ala Ala Val Arg Ile Tyr Gly His
35 40 45

Thr Glu Thr Thr Trp Cys Cys Ala Cys Tyr Glu Leu Thr Phe Thr Ser
50 55 60

Gly Pro Val Ser Gly Lys Lys Met Ile Val Gln
65 70 75

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Fusarium oxysporum ssp lycopersici
(B) STRAIN: CBS 645.78

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..177

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

AGA AAC GAC AAC CCC ATC TCC AAC ACC AAC GCT GTC AAC GGT TGT GAG 48
Arg Asn Asp Asn Pro Ile Ser Asn Thr Asn Ala Val Asn Gly Cys Glu
30 35 40 45

GGT GGT GGT TCT GCT TAT GCT TGC ACC AAC TAC TCT CCC TGG GCT GTC 96
Gly Gly Gly Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro Trp Ala Val
50 55 60

AAC GAT GAG CTT GCC TAC GGT TTC GCT GCT ACC AAG ATC TCC GGT GGC 144
Asn Asp Glu Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile Ser Gly Gly

65 70 75

TCC GAG GCC AGC TGG TGC TGT GCC TGC TAT CTA
177

Ser Glu Ala Ser Trp Cys Cys Ala Cys Tyr Leu
80 85

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Arg Asn Asp Asn Pro Ile Ser Asn Thr Asn Ala Val Asn Gly
Cys Glu
1 5 10
15

Gly Gly Gly Ser Ala Tyr Ala Cys Thr Asn Tyr Ser Pro Trp
Ala Val
20 25 30

Asn Asp Glu Leu Ala Tyr Gly Phe Ala Ala Thr Lys Ile Ser
Gly Gly
35 40 45

Ser Glu Ala Ser Trp Cys Cys Ala Cys Tyr Leu
50 55

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nectria pinea
(B) STRAIN: CBS 279.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..63

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

AGC GGC TGT GAC GGT GGT TCT GCC TAC GCC TGT GCA AAC AAC
TCC CCT 48
Ser Gly Cys Asp Gly Gly Ser Ala Tyr Ala Cys Ala Asn Asn
Ser Pro
60 65 70
75

TGG GCT GTC AAC GAT
63
Trp Ala Val Asn Asp
80

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Ser Gly Cys Asp Gly Gly Ser Ala Tyr Ala Cys Ala Asn Asn Ser Pro
1 5 10 15

Trp Ala Val Asn Asp
20

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola grisea Traeen
(B) STRAIN: ATCC 22726

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..177

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

AAC CAG CCT GTC TTC ACT TGC GAC GCC AAA TTC CAG CGC ATC ACC GAC 48
Asn Gln Pr Val Phe Thr Cys Asp Ala Lys Phe Gln Arg Ile

Thr Asp
25 30 35

CCC AAT ACC AAG TCG GGC TGC GAT GGC GGC TCG GCC TTT TCG
TGT GCT 96
Pro Asn Thr Lys Ser Gly Cys Asp Gly Gly Ser Ala Phe Ser
Cys Ala
40 45 50

GAC CAA ACC CCC TGG GCT CTG AAC GAC GAT TTC GCC TAT GGC
TTC GCT 144
Asp Gln Thr Pro Trp Ala Leu Asn Asp Asp Phe Ala Tyr Gly
Phe Ala
55 60 65

GCC ACG GCT ATT TCG GGT GGA TCG GAA GCC TCG
177
Ala Thr Ala Ile Ser Gly Gly Ser Glu Ala Ser
70 75 80

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Asn Gln Pro Val Phe Thr Cys Asp Ala Lys Phe Gln Arg Ile
Thr Asp
1 5 10

15

Pro Asn Thr Lys Ser Gly Cys Asp Gly Gly Ser Ala Phe Ser Cys Ala
20 25 30

Asp Gln Thr Pro Trp Ala Leu Asn Asp Asp Phe Ala Tyr Gly Phe Ala
35 40 45

Ala Thr Ala Ile Ser Gly Gly Ser Glu Ala Ser
50 55

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Humicola nigrescens Omvik
(B) STRAIN: CBS 819.73

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GTC TAC GCC TGC AAC GCA AAC TTC CAG CGC ATC ACC GAC GCC

AAC GCC 48
Val Tyr Ala Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Ala
Asn Ala
60 65 70
75

AAG TCC GGC TGC GAT GGC GGC TCC GCC TTC TCG TGC GCC AAC
CAG ACC 96
Lys Ser Gly Cys Asp Gly Gly Ser Ala Phe Ser Cys Ala Asn
Gln Thr
80 85
90

CCG TGG GCC GTG AGC GAC GAC TTT GCC TAC GGT TTC GCG GCT
ACG GCG 144
Pro Trp Ala Val Ser Asp Asp Phe Ala Tyr Gly Phe Ala Ala
Thr Ala
95 100 105

CTC GCC GGC
153
Leu Ala Gly
110

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Tyr Ala Cys Asn Ala Asn Phe Gln Arg Ile Thr Asp Ala

Asn Ala
1 5 10
15

Lys Ser Gly Cys Asp Gly Gly Ser Ala Phe Ser Cys Ala Asn
Gln Thr
20 25 30

Pro Trp Ala Val Ser Asp Asp Phe Ala Tyr Gly Phe Ala Ala
Thr Ala
35 40 45

Leu Ala Gly
50

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Cladorrhinum foecundissimum
(B) STRAIN: ATCC 62373

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..181

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GTC AAC CGC CCT GTC CTC GCC TGC GAC GCA AAC AAC AAC CCT
CTG ACC 48
Val Asn Arg Pro Val Leu Ala Cys Asp Ala Asn Asn Asn Pro
Leu Thr
1 5 10
15

GAC GCC GGC GTC AAG TCC GGA TGT GAT GGC GGT TCT GCA TAC
ACC TGT 96
Asp Ala Gly Val Lys Ser Gly Cys Asp Gly Gly Ser Ala Tyr
Thr Cys
20 25 30

GCC AAC AAC TCC CCA TGG GCA GTG AAC GAC CAG CTC GCC TAC
GGC TTT 144
Ala Asn Asn Ser Pro Trp Ala Val Asn Asp Gln Leu Ala Tyr
Gly Phe
35 40 45

GCC GCC ACC AAA CTG AGC GGC GGA ACT GAG TCG TCA
180
Ala Ala Thr Lys Leu Ser Gly Gly Thr Glu Ser Ser
50 55 60

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Val Asn Arg Pro Val Leu Ala Cys Asp Ala Asn Asn Asn Pro
Leu Thr
1 5 10
15

Asp Ala Gly Val Lys Ser Gly Cys Asp Gly Gly Ser Ala Tyr
Thr Cys
20 25 30

Ala Asn Asn Ser Pro Trp Ala Val Asn Asp Gln Leu Ala Tyr
Gly Phe
35 40 45

Ala Ala Thr Lys Leu Ser Gly Gly Thr Glu Ser Ser
50 55 60

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Syspastospora boninensis
(B) STRAIN: NKBC 1515

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..64

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GGC TGC GAC GGC GGC AGC GCC TTC ACC TGC TCC AAC AAC TCT
CCA TGG 48
Gly Cys Asp Gly Gly Ser Ala Phe Thr Cys Ser Asn Asn Ser
Pro Trp

GCT GTG AAC GAA GAT
63
Ala Val Asn Glu Asp

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Gly Cys Asp Gly Gly Ser Ala Phe Thr Cys Ser Asn Asn Ser
Pro Trp

Ala Val Asn Glu Asp

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 153 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Nigrospora sp
(B) STRAIN: CBS 272.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```
ACA AGA AAC GAC GGG CCC CTG TCC AGC CCC GAT GCC GCC TCC
GGC TGT          48
Thr Arg Asn Asp Gly Pro Leu Ser Ser Pro Asp Ala Ala Ser
Gly Cys
               25                  30                  35


GAT GGC GGC GAA GCC TTT GCC TGT TCT AAT ACC TCG CCT TGG
GCC GTC          96
Asp Gly Gly Glu Ala Phe Ala Cys Ser Asn Thr Ser Pro Trp
Ala Val
           40                  45                  50


AGC GAC CAG CTC GCG TAC GGA TAC GTC GCC ACG TCC ATC TCC
GGC GGC         144
Ser Asp Gln Leu Ala Tyr Gly Tyr Val Ala Thr Ser Ile Ser
Gly Gly
      55                  60                  65
```

ACC GAG TCA
153
Thr Glu Ser
70

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Thr Arg Asn Asp Gly Pro Leu Ser Ser Pro Asp Ala Ala Ser Gly Cys
1 5 10 15

Asp Gly Gly Glu Ala Phe Ala Cys Ser Asn Thr Ser Pro Trp Ala Val
20 25 30

Ser Asp Gln Leu Ala Tyr Gly Tyr Val Ala Thr Ser Ile Ser Gly Gly
35 40 45

Thr Glu Ser
50

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Chaetostylum fresenii

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..159

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GTC CGA ACG TGT AGT GCC AAC GAC TCG CCC TTG TCC GAC CCA AAT GCC 48
Val Arg Thr Cys Ser Ala Asn Asp Ser Pro Leu Ser Asp Pro Asn Ala
55 60 65

CCA AGT GGG TGT GAC GGT GGT AGC GCC TTC ACT TGT TCC AAC AAC TCC 96
Pro Ser Gly Cys Asp Gly Gly Ser Ala Phe Thr Cys Ser Asn Asn Ser
70 75 80

CCG TGG GCA GTC GAT GAC CAG ACA GCT TAT GGC TTT GCG GCA ACA GCC 144
Pro Trp Ala Val Asp Asp Gln Thr Ala Tyr Gly Phe Ala Ala Thr Ala
85 90 95

ATC AGT GGC CAG TCC
159
Ile Ser Gly Gln Ser
100

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Val Arg Thr Cys Ser Ala Asn Asp Ser Pro Leu Ser Asp Pro
Asn Ala
1 5 10
15

Pro Ser Gly Cys Asp Gly Gly Ser Ala Phe Thr Cys Ser Asn
Asn Ser
20 25 30

Pro Trp Ala Val Asp Asp Gln Thr Ala Tyr Gly Phe Ala Ala
Thr Ala
35 40 45

Ile Ser Gly Gln Ser
50

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 153 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Exidia glandulosa
(B) STRAIN: CBS 277.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..153

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

TGT GAG AAG AAC GAC AAC CCC TTA GCT GAC TTC AGC ACG AAA
TCC GGG 48
Cys Glu Lys Asn Asp Asn Pro Leu Ala Asp Phe Ser Thr Lys
Ser Gly
55 60 65

TGT GAA AGC GGA GGT TCG GCT TAT ACG TGT AAC AAC CAA TCA
CCA TGG 96
Cys Glu Ser Gly Gly Ser Ala Tyr Thr Cys Asn Asn Gln Ser
Pro Trp
70 75 80
85

GCC GTC AAT GAC TTG GTG TCG TAT GGC TTC GCC GCC ACA GCG
ATC AAT 144
Ala Val Asn Asp Leu Val Ser Tyr Gly Phe Ala Ala Thr Ala
Ile Asn
90 95

100

GGT GGC AAT
153
Gly Gly Asn

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Cys Glu Lys Asn Asp Asn Pro Leu Ala Asp Phe Ser Thr Lys
Ser Gly
1 5 10
15

Cys Glu Ser Gly Gly Ser Ala Tyr Thr Cys Asn Asn Gln Ser
Pro Trp
20 25 30

Ala Val Asn Asp Leu Val Ser Tyr Gly Phe Ala Ala Thr Ala
Ile Asn
35 40 45

Gly Gly Asn
50

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 171 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Coniothecium sp

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..171

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
AGC CGC CCC GTC GGA ACC TGC AAG AGG AAC GAC AAC CCC CTC
TCC GAC          48
Ser Arg Pro Val Gly Thr Cys Lys Arg Asn Asp Asn Pro Leu
Ser Asp
                 55                  60                  65

CCC GAT GCC AAG TCC GGC TGC GAC GGC GGC GGC GCC TTC ATG
TGC TCC          96
Pro Asp Ala Lys Ser Gly Cys Asp Gly Gly Gly Ala Phe Met
Cys Ser
            70                  75                  80

ACC CAG CAG CCG TGG GCC GTC AAC GAC AAT CTG GCA TAT GGC
TTC GCC         144
Thr Gln Gln Pro Trp Ala Val Asn Asp Asn Leu Ala Tyr Gly
Phe Ala
        85                  90                  95
```

GCC ACG GCC ATC AGC GGC GGC AAC GAG
171

Ala Thr Ala Ile Ser Gly Gly Asn Glu
100 105

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Ser Arg Pro Val Gly Thr Cys Lys Arg Asn Asp Asn Pro Leu
Ser Asp
1 5 10
15

Pro Asp Ala Lys Ser Gly Cys Asp Gly Gly Gly Ala Phe Met
Cys Ser
20 25 30

Thr Gln Gln Pro Trp Ala Val Asn Asp Asn Leu Ala Tyr Gly
Phe Ala
35 40 45

Ala Thr Ala Ile Ser Gly Gly Asn Glu
50 55

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: CBS 271.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..159

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
ACT TGC AAC AAG AAC GAC GGG CCC CTG TCC AGC CCC GAT GCC
GCC TCC          48
Thr Cys Asn Lys Asn Asp Gly Pro Leu Ser Ser Pro Asp Ala
Ala Ser
          60                  65                  70

GGC TGT GAT GGC GGC GAA GCC TTT GCC TGT TCT AAT ACC TCG
CCT TGG          96
Gly Cys Asp Gly Gly Glu Ala Phe Ala Cys Ser Asn Thr Ser
Pro Trp
     75                  80                  85

GCC GTC AGC GAC CAG CTC GCG TAC GGA TAC CTC GCC ACG TCC
ATC TCC         144
Ala Val Ser Asp Gln Leu Ala Tyr Gly Tyr Leu Ala Thr Ser
Ile Ser
 90                  95                  100
```

105

GGC GGC ACC GAG TCG
159
Gly Gly Thr Glu Ser
110

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Thr Cys Asn Lys Asn Asp Gly Pro Leu Ser Ser Pro Asp Ala
Ala Ser
1 5 10
15

Gly Cys Asp Gly Gly Glu Ala Phe Ala Cys Ser Asn Thr Ser
Pro Trp
20 25 30

Ala Val Ser Asp Gln Leu Ala Tyr Gly Tyr Leu Ala Thr Ser
Ile Ser
35 40 45

Gly Gly Thr Glu Ser
50

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: CBS 270.96

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..84

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CCA GTT TTC TCC TGT GAC AAG TAC GAC AAC CCT CTA CCT GAC GCC AAT 48
Pro Val Phe Ser Cys Asp Lys Tyr Asp Asn Pro Leu Pro Asp Ala Asn
55 60 65

GCT GTG TCC GGG TGT GAC CCC GGA GGT ACT GCC TTC 84
Ala Val Ser Gly Cys Asp Pro Gly Gly Thr Ala Phe
70 75 80

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Pro Val Phe Ser Cys Asp Lys Tyr Asp Asn Pro Leu Pro Asp
Ala Asn
1 5 10
15

Ala Val Ser Gly Cys Asp Pro Gly Gly Thr Ala Phe
20 25

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Diplodia gossypina, CBS 274.96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

ACCTGCGACG CCTGCGACAG CCCCCTCAGC GACTACGACG CCAAGTCCGG
CTGCGACGGC 60

GGTAGCGCAT ACACCTGCAC CTACTCTACC CCCTGGGCCG TCGACGACAA
CCTCTCCTAC 120

GGTTTCGCCG CCGCCAAGCT GAGCGGA
147

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Thr Cys Asp Ala Cys Asp Ser Pro Leu Ser Asp Tyr Asp Ala Lys Ser
1 5 10 15

Gly Cys Asp Gly Gly Ser Ala Tyr Thr Cys Thr Tyr Ser Thr Pro Trp
20 25 30

Ala Val Asp Asp Asn Leu Ser Tyr Gly Phe Ala Ala Ala Lys Leu Ser
35 40 45

Gly

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Ulospora bilgramii, NKBC 1444

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CCACTAGCAG ATTTCACCGG TGGAACCGGC TGTAATGGCG GTTCGACATT CTCATGCTCA 60

AACCAACAAC CATGGGCGGT CAACGACACA TTCTCGTACG GCTTTGCGGG CATCTTTATC 120

ACAGGCCATG TCGAG 135

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Pro Leu Ala Asp Phe Thr Gly Gly Thr Gly Cys Asn Gly Gly Ser Thr
1 5 10

15

Phe Ser Cys Ser Asn Gln Gln Pro Trp Ala Val Asn Asp Thr Phe Ser

20 25 30

Tyr Gly Phe Ala Gly Ile Phe Ile Thr Gly His Val Glu
35 40 45

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Penicillium verruculosum, ATCC 62396

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GCCAAATCTG GATGTGATGC TGGTGGAGGT CAAGCCTACA TGTGCTCCAA CCAACAACCT 60

TGGGTAGTCA ACGACAACCT CGCCTACGGT TTCGCCGCAG TCAACATTGC CGGC 114

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Ala Lys Ser Gly Cys Asp Ala Gly Gly Gly Gln Ala Tyr Met Cys Ser
1 5 10 15

Asn Gln Gln Pro Trp Val Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala
20 25 30

Ala Val Asn Ile Ala Gly
35

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Poronia punctata

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

TTCGACGTCC GGGTGCGACA ATGGCGGCAG CGCCTTCATG TGCTCTAACC AAAGCCCCTG 60

GGCCGTCAAC GACGATCTGG CCTACGGCTG GGCCGCCGTC TCAATCGCGG GCC 113

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Ser Thr Ser Gly Cys Asp Asn Gly Gly Ser Ala Phe Met Cys Ser Asn
1 5 10 15

Gln Ser Pro Trp Ala Val Asn Asp Asp Leu Ala Tyr Gly Trp Ala Ala
20 25 30

Val Ser Ile Ala Gly
35

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 177 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Fusarium anguioides, IFO 4467

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

TCAACACCGG TGCAGACGTG CGACCGCAAC GACAACCCGC TCTACGACGG CGGGTCGACG 60

CGGTCCGGCT GCGACGCCGG CGGCGGCGCC TACATGTGCT CGTCGCACAG CCCGTGGGCC 120

GTCAGCGACA GCCTCTCGTA CGGCTGGGCG GCCGTCCGCA TCGCCGGCCA GTCCGAG 177

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Ser Thr Pro Val Gln Thr Cys Asp Arg Asn Asp Asn Pro Leu Tyr Asp
1 5 10 15

Gly Gly Ser Thr Arg Ser Gly Cys Asp Ala Gly Gly Gly Ala Tyr Met
20 25 30

Cys Ser Ser His Ser Pro Trp Ala Val Ser Asp Ser Leu Ser Tyr Gly
35 40 45

Trp Ala Ala Val Arg Ile Ala Gly Gln Ser Glu
50 55

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 150 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Thielavia thermophila, CBS 174.70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

AACGACAACC CCATCTCCAA CACCAACGCT GTCAACGGTT GTGAGGGTGG

TGGTTCTGCT 60

TACGCTTGCT CCAACTACTC TCCCTGGGCT GTCAACGATG ACCTTGCCTA CGGTTTCGCT 120

GTTACCAAGA TCTCCGGTGG CTCCGAGGCC 150

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Asn Asp Asn Pro Ile Ser Asn Thr Asn Ala Val Asn Gly Cys Glu Gly
1 5 10 15

Gly Gly Ser Ala Tyr Ala Cys Ser Asn Tyr Ser Pro Trp Ala Val Asn
20 25 30

Asp Asp Leu Ala Tyr Gly Phe Ala Val Thr Lys Ile Ser Gly Gly Ser
35 40 45

Glu Ala
50

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 180 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Chaetomium cuniculorum, CBS 799.83

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

GTCAATCAGC CCATCCGAAC GTGTAGTGCC AACGACTCGC CCTTGTCCGA CCCAAATACC 60

CCAAGTGGCT GTGACGGTGG TAGCGCCTTC ACTTGTTCCA ACAACTCCCC GTGGGCAGTC 120

GATGACCAGA CAGCTTATGG CTTTGCGGCA ACAGCCATCA GTGGCCAGTC CGAGAGCAGC 180

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

Val Asn Gln Pro Ile Arg Thr Cys Ser Ala Asn Asp Ser
Pro Leu Ser
1 5 10
15

Asp Pro Asn Thr Pro Ser Gly Cys Asp Gly Gly Ser Ala
Phe Thr Cys
20 25
30

Ser Asn Asn Ser Pro Trp Ala Val Asp Asp Gln Thr Ala
Tyr Gly Phe
35 40 45

Ala Ala Thr Ala Ile Ser Gly Gln Ser Glu Ser Ser
50 55 60

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 159 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ORIGINAL SOURCE: Chaetomium virescens, CBS 547.75

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

ACCTGCGACA AGAAGGACAA CCCCATCTCT GATGCCAACG CCAAGAGCGG CTGTGATGGC 60

GGTTCTGCTT TCGCCTGCAC CAACTACTCT CCCTTCGCCG TCAACGACAA CCTCGCCTAC 120

GGTTTCGCTG CCACCAAGCT TGCTGGAGGC TCCGAGGCT 159

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

Thr Cys Asp Lys Lys Asp Asn Pro Ile Ser Asp Ala Asn Ala Lys Ser
1 5 10 15

Gly Cys Asp Gly Gly Ser Ala Phe Ala Cys Thr Asn Tyr Ser Pro Phe
20 25 30

Ala Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala Ala Thr Lys Leu Ala

35 40 45

Gly Gly Ser Glu Ala
50

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Colletotrichum lagenarium

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

ACCTGCTACG CCAATGACCA GCGCATCGCC GACCGCAGCA CCAAGTCCGG CTGCGACGGC 60

GGCTCGGCCT ACTCCTGTTC T
81

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

Thr Cys Tyr Ala Asn Asp Gln Arg Ile Ala Asp Arg Ser
Thr Lys Ser
1 5 10
15

Gly Cys Asp Gly Gly Ser Ala Tyr Ser Cys Ser
20 25

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 160 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(B) STRAIN: Phycomyces nitens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

ACCTGTGACA AGAAGGACAA CCCCATCTCA AACTTGAACG CTGTCAACGG
TTGTGAGGGT 60

GGTGGTTCTG CCTTCGCCTG CACCAACTAC TCTCCTTGGG CGGTCAATGA
CAACCTTGCC 120

TACGGCTTCG CTGCAACCAA GCTTGCCGGT GGCTCCGAGG
160

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

Thr Cys Asp Lys Lys Asp Asn Pro Ile Ser Asn Leu Asn Ala Val Asn
1 5 10 15

Gly Cys Glu Gly Gly Gly Ser Ala Phe Ala Cys Thr Asn Tyr Ser Pro
20 25 30

Trp Ala Val Asn Asp Asn Leu Ala Tyr Gly Phe Ala Ala Thr Lys Leu
35 40 45

Ala Gly Gly Ser Glu
50

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ORIGINAL SOURCE: Trichothecium roseum, IFO 5372

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

CCAGTAGGCA CCTGCGACGC CGGCAACAGC CCCCTCGGCG ACCCCCTGGC CAAGTCTGGC 60

TGCGAGGGCG GCCCGTCGTA CACGTGCGCC AACTACCAGC CGTGGGCGGT CAACGACCAG 120

CTGGCCTACG GCTTCGCGGC CACGGCCATC AACGGCGGCA CCGAG 165

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

Pro Val Gly Thr Cys Asp Ala Gly Asn Ser Pro Leu Gly Asp Pro Leu
1 5 10 15

Ala Lys Ser Gly Cys Glu Gly Gly Pro Ser Tyr Thr Cys
Ala Asn Tyr
20 25
30

Gln Pro Trp Ala Val Asn Asp Gln Leu Ala Tyr Gly Phe
Ala Ala Thr
35 40 45

Ala Ile Asn Gly Gly Thr Glu
50 55

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12 March 1996	CBS 273.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer: c.A. S. Henriksson	Authorized officer:

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 18, line 35.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 274.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer: S. Henderson	Authorized officer:

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 18, line 37.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 275.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer A. G. Henriksen	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 17, line 70.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 276.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer C. S. Henriksen	Authorized officer

Form PCT/RO/134 (July 1992)

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 17, line 9.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 277.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer S. Henderson	Authorized officer

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 27.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 278.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer Anne-G... H...ksson H... ...	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 20, line 37.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 279.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 17, line 10.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 280.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 18, line 36.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 281.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 17, line 34.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 282.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 17, line 11.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 283.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 28.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 284.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 2.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
12 March 1996	CBS 285.96

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer:	Authorized officer:

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 21, line 7.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
CENTRAALBUREAU VOOR SCHIMMELCULTURES

Address of depositary institution *(including postal code and country)*
Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, the Netherlands

Date of deposit	Accession Number
28. September, 1994	CBS 478.94

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 22, line 37.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
24 February 1995	DSM 9770

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

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E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 22, line 37.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
30 June 1995	DSM 10080

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 22, line 37.	
B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet [x]
Name of depositary institution DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH	
Address of depositary institution *(including postal code and country)* Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany	
Date of deposit 30 June 1995	Accession Number DSM 10081
C. ADDITIONAL INDICATIONS *(leave blank if not applicable)*	This information is continued on an additional sheet [ ]
During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*	
E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*	
The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*	

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Authorized officer	Authorized officer

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 22, line 37.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
30 June 1995	DSM 10082

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 7.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
2 February 1996	DSM 10511

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 1.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
6 March 1996	DSM 10571

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 7.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [x]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
2 February 1996	DSM 10512

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 23, line 1.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution (*including postal code and country*)
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
12 March 1996	DSM 10576

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 24.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10583

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 29.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10584

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 24, line 34.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10585

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 25, line 7.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10586

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 25, line 6.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution

DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*

Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10587

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 25, line 11.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [x]

Name of depositary institution
DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELL-KULTUREN GmbH

Address of depositary institution *(including postal code and country)*
Mascheroder Weg 1b, D-38124 Braunschweig, Federal Republic of Germany

Date of deposit	Accession Number
13 March 1996	DSM 10588

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

During the pendency of the patent application a sample of the deposited microorganism is only to be provided to an independent expert nominated by the person requesting the sample (cf. e.g. Rule 28(4) EPC / Regulation 3.25 of Australia Statutory Rules 1991 No 71) in those designated states providing for such "expert solution".

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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CLAIMS

1. An enzyme preparation consisting essentially of an enzyme which has cellulytic activity and comprises a first amino acid sequence consisting of 14 amino acid residues having the following sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp	Xaa
1	2	3	4	5	6	7	8	9	10	11	12	13	14

and a second amino acid sequence consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, 12 and 14, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues with the provisos that, in the first amino acid sequence, (i) when the amino residue in position 12 is Ser, then the amino acid residue in position 14 is not Ser, and (ii) when the amino residue in position 12 is Gly, then the amino acid residue in position 14 is not Ala.

2. The enzyme preparation according to claim 1, wherein the amino acid residue in position 9 of the first sequence is selected from the group consisting of

proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of proline and threonine.

3. The enzyme preparation according to claim 1 or 2, wherein the amino acid residue in position 10 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably serine.

4. The enzyme preparation according to any of the claims 1-3, wherein the amino acid residue in position 12 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of alanine and glycine.

5. The enzyme preparation according to any of the claims 1-4, wherein the amino acid residue in position 14 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine, tryptophan, glutamic acid and aspartic acid, preferably from the group consisting of proline, threonine, serine, alanine, glutamic acid and aspartic acid.

6. The enzyme preparation according to any of the claims 1-5, wherein the amino acid residue in position 4 of the second sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methi nine, tryptophan, glutamic acid

and aspartic acid, preferably from the group consisting of alanine, glycine, and glutamine.

7. The enzyme preparation according to any of the claims 1-6, wherein, in the first sequence, the amino acid residue in position 3 is tyrosine; or the amino acid residue in position 4 is tryptophan; or the amino acid residue in position 8 is lysine.

8. The enzyme preparation according to any of the claims 1-7, wherein the first sequence comprises an amino acid sequence selected from the group consisting of the sequences

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	;

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Thr	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	; and

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Gly	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	.

9. The enzyme preparation according to any of the claims 1-8 which is of microbial origin, preferably fungal origin.

10. A DNA construct encoding for the enzyme according to any of the claims 1-9.

11. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to *Hymenomycetes (Basidiomycota)* which enzyme comprises an amino acid sequence selected from the group consisting of the sequences

Xaa Thr Arg Xaa Phe Asp Xaa

1 2 3 4 5 6 7 ;

Xaa Thr Arg Xaa Tyr Asp Xaa
1 2 3 4 5 6 7 ; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

12. The enzyme preparation according to claim 11, wherein the amino acid residue in position 7 is cysteine (Cys).

13. The enzyme preparation according to claim 11, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

14. The enzyme preparation according to any of the claims 11-13, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

15. The enzyme preparation according to any of the claims 11-14 wherein the enzyme is obtainable from a strain belonging to the group consisting of the orders *Agaricales, Aphyllophorales,* and *Auriculariales*.

16. The enzyme preparation according to claim 15 wherein the enzyme is obtainable from a strain belonging to the group consisting of the families *Exidiaceae, Tricholomataceae, Coprinaceae, Schizophyllaceae, Bjerkanderaceae* and *Polyporaceae*, preferably belonging to the group consisting of the genera *Exidia, Crinipellis, Fomes, Panaeolus, Trametes, Schizophyllum,* and *Spongipellis*.

17. The enzyme preparation according to claim 16 wherein the enzyme is obtainable from a strain belonging to the group consisting of the species *Exidia glandulosa, Crinipellis scabella, Fomes fomentarius,* and *Spongipellis sp.*, preferably from *Exidia glandulosa,* CBS 277.96, *Crinipellis scabella,* CBS 280.96, *Fomes fomentarius,* CBS 276.96, and *Spongipellis sp.*, CBS 283.96.

18. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to *Chytridiomycota* which enzyme comprises an amino acid sequence selected from the group

consisting of the sequences

Xaa Thr Arg Xaa Phe Asp Xaa
1 2 3 4 5 6 7 ;

Xaa Thr Arg Xaa Tyr Asp Xaa
1 2 3 4 5 6 7 ; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

19. The enzyme preparation according to claim 18, wherein the amino acid residue in position 7 is cysteine (Cys).

20. The enzyme preparation according to claim 18, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

21. The enzyme preparation according to any of the claims 18-20, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

22. The enzyme preparation according to any of the claims 18-21 wherein the enzyme is obtainable from a strain belonging to the class of *Chytridiomycetes*, preferably belonging to the group consisting of the orders *Chytridiales, Spizellomycetales, Harpochytriales,* and *Blastocladiales*.

23. The enzyme preparation according to claim 22 wherein the enzyme is obtainable from a strain belonging the family *Spizellomycetaceae*, preferably belonging to the genus *Rhizophlyctis*, preferably belonging to the species *Rhizophlyctis rosea, especially R.. rosea.,* CBS 282.96.

24. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to *Zygomycota* which enzyme comprises an amino acid sequence selected from the group consisting of the sequences

Xaa	Thr	Arg	Xaa	Phe	Asp	Xaa	
1	2	3	4	5	6	7	;

Xaa	Thr	Arg	Xaa	Tyr	Asp	Xaa	
1	2	3	4	5	6	7	; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein, in position 4, Xaa is Trp, Tyr or Phe; and in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

25. The enzyme preparation according to claim 24, wherein the amino acid residue in position 7 is cysteine (Cys).

26. The enzyme preparation according to claim 24, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

27. The enzyme preparation according to any of the claims 24-26, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is

Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

28. The enzyme according to any of the claims 24-27 which enzyme is obtainable from a strain belonging to the class *Zygomycetes*, preferably to the order *Mucorales*.

29. The enzyme according to claim 28 which enzyme is obtainable from a strain belonging to the group consisting of the families *Mucoracea* and *Thamnidiaceae*, preferably belonging to the group consisting of the genera *Rhizomucor*, *Phycomyces and Chaetostylum*.

30. The enzyme according to claim 29 which enzyme is obtainable from a strain belonging the group consisting of the species *Rhizomucor pusillus*, *Phycomyces nitens*, *Chaetostylum fresenii*, preferably *Rhizomucor pusillus*, IFO 4578, *Phycomyces nitens*, IFO 4814, and *Chaetostylum fresenii*, NRRL 2305.

31. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to the group consisting of *Archaeascomycetes*, *Discomycetes*, *Hermiascomycetes*, *Loculoascomycetes*, *and Plectomycetes* which enzyme comprises an amino acid sequence selected from the group consisting of the sequences

Xaa	Thr	Arg	Xaa	Phe	Asp	Xaa	
1	2	3	4	5	6	7	;

Xaa	Thr	Arg	Xaa	Tyr	Asp	Xaa	
1	2	3	4	5	6	7	; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

32. The enzyme preparation according to claim 31, wherein the amino acid residue in position 7 is cysteine (Cys).

33. The enzyme preparation according to claim 31, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

34. The enzyme preparation according to any of the claims 31-33, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is

Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

35. The enzyme preparation according to any of the claims 31-34 wherein the enzyme is obtainable from a strain belonging to the group consisting of the orders *Pezizales, Phytismatales, Dothideales,* and *Eurotiales.*

36. The enzyme preparation according to claim 35 wherein the enzyme is obtainable from a strain belonging the the group consisting of the families *Cucurbitariaceae, Rhytismataceae, Ascobolaceae,* and *Trichocomaceae,* preferably belonging the the group consisting of the genera *Diplodia, Microsphaeropsis, Ulospora, Macrophomina, Ascobolus, Saccobolus, Penicillium,* and *Thermomyces.*

37. The enzyme preparation according to claim 36 wherein the enzyme is obtainable from a strain belonging the the group consisting of the species *Diplodia gossypina, Microsphaeropsis sp., Ulospora bilgramii, Macrophomina phaseolina, Ascobolus stictoides, Saccobolus dilutellus, Penicillium verruculosum, Penicillium chrysogenum,* and *Thermomyces verrucosus;* preferably *Diplodia gossypina, CBS 274.96, Ulospora bilgramii, NKBC 1444, Macrophomina phaseolina, CBS 281.96, Saccobolus dilutellus, CBS 275.96, Penicillium verruculosum, ATCC 62396, Penicillium chrysogenum,* ATCC 9480, and *Thermomyces verrucosus*, CBS 285.96.

38. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to the group consisting of the orders *Diaportales, Xylariales, Trichoaphaeriales* and *Phyllachorales* which enzyme comprises an amino acid

sequence selected from the group consisting of the sequences

Xaa Thr Arg Xaa Phe Asp Xaa
1 2 3 4 5 6 7 ;

Xaa Thr Arg Xaa Tyr Asp Xaa
1 2 3 4 5 6 7 ; and

Xaa Thr Arg Xaa Trp Asp Xaa
1 2 3 4 5 6 7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

39. The enzyme preparation according to claim 38, wherein the amino acid residue in position 7 is cysteine (Cys).

40. The enzyme preparation according to claim 38, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

41. The enzyme preparation according to any of the claims 38-40, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr Arg Xaa Xaa Asp Cys Cys Xaa Xaa Xaa Cys Xaa Trp
1 2 3 4 5 6 7 8 9 10 11 12 13

and a second peptide consisting of 5 amino acid residues having the following sequence
Trp Cys Cys Xaa Cys
1 2 3 4 5

wherein,
in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

42. The enzyme preparation according to any of the claims 38-41 wherein the enzyme is obtainable from a strain belonging to the group consisting of the families *Xylariaceae, Valsaceae,* and *Phyllachoraceae*, preferably belonging to the genera *Diaporthe, Colletotrichum, Nigrospora, Xylaria, Nodulisporum* and *Poronia*.

43. The enzyme according to claim 42 which enzyme is obtainable from a strain belonging to the group consisting of the species *Diaporthe syngenesia, Colletotrichum lagenarium, Nigrospora sp., Xylaria hypoxylon, Nodulisporum sp.,* and *Poronia punctata,* preferably *Diaporthe syngenesia, CBS 278.96, Colletotrichum lagenarium, ATCC 52609, Nigrospora sp., CBS 272.96, Xylaria hypoxylon, CBS 284.96*

44. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to the group consisting of the families *Nectriaceae, Sordariaceae, Chaetomiaceae, Ceratostomaceae, Lasiosphaeriaceae* and the genera *Acremonium, Gliocladium, Scytalidium, Cylindrocarpon* and *Volutella* which enzyme comprises an amino acid sequence selected from the group consisting of the sequences

Xaa	Thr	Arg	Xaa	Phe	Asp	Xaa	
1	2	3	4	5	6	7	;

Xaa	Thr	Arg	Xaa	Tyr	Asp	Xaa	
1	2	3	4	5	6	7	; and

Xaa	Thr	Arg	Xaa	Trp	Asp	Xaa
1	2	3	4	5	6	7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

45. The enzyme preparation according to claim 44, wherein the amino acid residue in position 7 is cysteine (Cys).

46. The enzyme preparation according to claim 44, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

47. The enzyme preparation according to any of the claims 44-46, wherein the enzyme comprises a first peptide consisting of 13 amino acid residues having the following sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp
1	2	3	4	5	6	7	8	9	10	11	12	13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein,

in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

48. The enzyme preparation according to any of the claims 44-47 wherein the enzyme is obtainable from a strain belonging to the group consisting of the genera *Cylindrocarpon, Nectria, Volutella, Sordaria, Thielavia, Sypastospora, Chaetomium, Myceliophthora, Scytalidium, Cladorrhinum, Gliocladium, Acremonium*.

49. The enzyme according to claim 48 which enzyme is obtainable from a strain belonging to the group consisting of the species *Cylindrocarpon sp., Nectria pinea, Volutella colletotrichoides, Sordaria fimicola, Sordaria macrospora, Thielavia terrestris, Thielavia thermophila, Syspastospora boninensis, Cladorrhinum foecundissimum, Chaetomium murorum, Chaetomium virescens, Chaetomium brasiliensis, Chaetomium cunicolorum, Myceliophthora thermophila, Gliocladium catenulatum, Scytalidium thermophila,* and *Acremonium sp.*, preferably from *Gliocladium catenulatum, ATCC 10523 & CBS 227.48, Nectria pinea, CBS 279.96, Volutella colletotrichoides,CBS 400.58, Sordaria fimicola, ATCC 52644, Sordaria macrospora, ATCC 60255, Thielavia terrestris, NRRL 8126, Thielavia thermophila, CCBS 174.70, Chaetomium murorum, CBS 163.52, Chaetomium virescens, CBS 547.75, Chaetomium brasiliensis, CBS 122.65, Chaetomium cunicolorum, CBS 799.83, Syspastospora boninensis, NKBC 1515, Cladorrhinum foecundissimum,* ATCC 62373, *Myceliophthora thermophila,*

CBS 117.65, *Scytalidium thermophila,* ATCC 28085, and *Acremonium sp.,* CBS 478.94

50. An enzyme preparation consisting essentially of an enzyme having cellulytic activity and being obtainable from a strain belonging to the group consisting of the species *Fusarium lycopersici, Fusarium passiflora, Fusarium solani, Fusarium anguioides, Fusarium poae, Humicola nigrescens* and *Humicola grisea* which enzyme comprises an amino acid sequence selected from the group consisting of the sequences

Xaa	Thr	Arg	Xaa	Phe	Asp	Xaa	
1	2	3	4	5	6	7	;

Xaa	Thr	Arg	Xaa	Tyr	Asp	Xaa	
1	2	3	4	5	6	7	; and

Xaa	Thr	Arg	Xaa	Trp	Asp	Xaa
1	2	3	4	5	6	7

wherein,
in position 4, Xaa is Trp, Tyr or Phe; and
in position 1 and 7, Xaa is any of the 20 naturally occurring amino acid residues.

51. The enzyme preparation according to claim 50, wherein the amino acid residue in position 7 is cysteine (Cys).

52. The enzyme preparation according to claim 50, wherein the amino acid residue in position 1 is selected from the group consisting of aspartic acid (Asp), threonine (Thr) and alanine (Ala).

53. The enzyme preparation according to any of the claims 50-52, wherein the enzyme comprises a first peptide c nsisting f 13 amino acid residues having the following

sequence

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp
1	2	3	4	5	6	7	8	9	10	11	12	13

and a second peptide consisting of 5 amino acid residues having the following sequence

Trp	Cys	Cys	Xaa	Cys
1	2	3	4	5

wherein,
in position 3 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 4 of the first sequence, the amino acid is Trp, Tyr or Phe;
in position 8 of the first sequence, the amino acid is Arg, Lys or His;
in position 9, 10, and 12, respectively, of the first sequence, and in position 4 of the second sequence, the amino acid is any of the 20 naturally occurring amino acid residues.

54. The enzyme preparation according to claim 53 wherein the enzyme is obtainable from a strain belonging to the group consisting of the strains *Fusarium oxysporum ssp lycopersici, CBS 645.78, Fusarium oxysporum ssp passiflora, CBS 744.79, Fusarium solani, IMI 107.511, Fusarium anguioides, IFO 4467, Fusarium poae, ATCC 60883, Humicola nigrescens*, CBS 819.73 and *Humicola grisea*, ATCC 22726.

55. The enzyme according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein the amino acid residue in position 9 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine,

glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of proline and threonine.

56. The enzyme according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein the amino acid residue in position 10 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably serine.

57. The enzyme according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein the amino acid residue in position 12 of the first sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine and tryptophan, preferably from the group consisting of alanine and glycine.

58. The enzyme according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein the amino acid residue in position 4 of the second sequence is selected from the group consisting of proline, threonine, valine, alanine, leucine, isoleucine, phenylalanine, glycine, cysteine, asparagine, glutamine, tyrosine, serine, methionine, tryptophan, glutamic acid and aspartic acid, preferably from the group consisting of alanine, glycine, and glutamine.

59. The enzyme according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein, in the first sequence, the amino acid residue in position 3 is tyrosine; or the amino acid residue in position 4 is tryptophan; or the amin acid r sidue in position 8 is

lysine.

60. A DNA construct encoding for the enzyme according to any of the claims 11-59.

61. The enzyme preparation according to any of the claims 14-17, 21-23, 27-30, 34-37, 41-43, 47-49, 53 and 54, wherein the first sequence comprises an amino acid sequence selected from the group consisting of the sequences

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	;

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Thr	Ser	Cys	Ala	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	; and

Thr	Arg	Tyr	Trp	Asp	Cys	Cys	Lys	Pro	Ser	Cys	Gly	Trp	
1	2	3	4	5	6	7	8	9	10	11	12	13	.

62. A method for providing a microbial strain comprising a gene encoding for the enzyme present in the enzyme preparation according to any of the claims 1-9, 11-59, and 61 which method comprises hybridization, e.g. PCR amplification, under standard conditions with an oligonucleotide derived from any of the conserved regions illustrated in Fig.1.

63. The method according to claims 62, wherein the oligonucleotide comprises a nucleotide sequence encoding at least a pentapeptide comprised in a peptide selected from the group consisting of

a.

Thr	Arg	Xaa	Xaa	Asp	Cys	Cys	Xaa	Xaa	Xaa	Cys	Xaa	Trp	Xaa
1	2	3	4	5	6	7	8	9	10	11	12	13	14

the amino acid in position 3 or 4 being Trp, Tyr or Phe; the amino acid in postion 8 being Arg, Lys or His;

the amino acid in position 9, 10, 12 and 14, respectively, being any of the 20 naturally occurring amino acid residues ; and

b.

Trp Cys Cys Xaa Cys Tyr
1 2 3 4 5 6

the amino acid in position 4 being any of the 20 naturally occurring amino acid residues ; and

c.

Xaa Pro Gly Gly Gly Xaa Gly Xaa Phe
1 2 3 4 5 6 7 8 9

the amino acid in position 1 being Met or Ile;
the amino acid in position 6 and 8, respectively, being Leu, Ile or Val; and

d.

Gly Cys Xaa Xaa Arg Xaa Asp Trp Xaa
1 2 3 4 5 6 7 8 9

the amino acid in position 3 being any of the 20 naturally occurring amino acid residues;
the amino acid in position 4 and 6, respectively, being Trp, Tyr or Phe; and
the amino acid in position 9 being Phe or Met;

64. The method according to claim 62, wherein the oligonucleotide comprises a nucleotide sequence complementary to the sequences of claim 63.

65. The method according to claim 63, wherein the oligonucleotide corresponds to a PCR primer selected from the group consisting of the PCR primers

sense,
5'-CCCCAAGCTTACI$^{A}/_{C}$GITA$^{C}/_{T}$TGGGA$^{C}/_{T}$TG$^{C}/_{T}$TG$^{C}/_{T}$AA$^{A}/_{O}$$^{A}/_{C}$C-3'
antisense 1,
5'- CTAGTCTAGATA$^{A}/_{O}$CAIGC$^{A}/_{O}$CA$^{A}/_{O}$CACC -3';
antisense 2,
5'- CTAGTCTAGAAAIA$^{A}/_{O}$/^{T}ICCIA^{A}/^{C}/^{O}ICCICCICCIGG -3'; and

antisense 3,
5'- CTAGTCTAGAIAACCA$^{A}/_{G}$TCA$^{A}/_{G}$$^{A}/_{T}AIC^{G}/_{T}$CC -3.

66. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting cellulytic activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 1, and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, or

b) an analogue of the DNA sequence shown in SEQ ID No. 1 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770, which

i) is homologous, preferably at least 70% homologous, with the DNA sequence shown in SEQ ID No. 1 and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 1 and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770,

iii) encodes a polypeptide which is homologous preferably at least 65% homologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 1 and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 9770,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 1 or obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 9770.

67. The DNA construct according to claim 66, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family

Chaetomiaceae, preferably to the genus *Myceliophthora*, in particular a strain of *M. thermophila*, especially *M. thermophila*, CBS 117.65.

68. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 4, and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10082, or

b) an analogue of the DNA sequence shown in SEQ ID No. 4 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10082, which

i) is homologous, preferably at least 70% homologous, with the DNA sequence shown in SEQ ID No. 4 and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10082,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 4 and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10082,

iii) encodes a polypeptide which is homologous preferably at least 60% homologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. and/or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10085,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 4 or obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 10082.

69. The DNA construct according to claim 68, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family

Hypocreaceae, preferably to the genus *Acremonium*, in particular *Acremonium sp.*, CBS 478.94.

70. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 6, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10080, or

b) an analogue of the DNA sequence shown in SEQ ID No. 6 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10080, which

i) is homologous, preferably 65% homologous, with the DNA sequence shown in SEQ ID No. 6 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10080,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 6 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10080,

iii) encodes a polypeptide which is homologous, preferably at least 70%, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 6 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10080,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 6 /or obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 10080.

71. The DNA construct according to claim 70, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family *Chaetomiceae*, preferably to the genus *Acremonium*, in par-

ticular *Acremonium sp.*, CBS 478.94.

72. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 8, or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10081, or

b) an analogue of the DNA sequence shown in SEQ ID No. 8 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10081, which

i) is homologous, preferably at least 75% homologous, with the DNA sequence shown in SEQ ID No. 8 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10081,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 8 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10081,

iii) encodes a polypeptide which is homologous, preferably at least 70% homologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 8 or the DNA sequence obtainable from the plasmid in *Saccharomyces cerevisiae* DSM 10081,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 8 or obtainable from the plasmid in *Saccharomyces cerevisiae*, DSM 10081.

73. The DNA construct according to claim 72, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family *Chaetomiaceae*, preferably to the genus *Thielavia*, in particular a strain of *Thielavia terrestris*, especially

Thielavia terrestris, NRRL 8126.

74. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 10, or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10512, or

b) an analogue of the DNA sequence shown in SEQ ID No. 10 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10512, which

i) is homologous, preferably at least 65% homologous, with the DNA sequence shown in SEQ ID No. 10 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10512,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 10 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10512,

iii) encodes a polypeptide which is homologous, preferably at least 55% homologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 10 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10512,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 10 or obtainable from the plasmid in *Escherichia coli*, DSM 10512.

75. The DNA construct according to claim 74, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family

Rhytismataceae, preferably to the genus *Macrophomina*, in particular *Macrophomina phaseolina, especially M.phaseolicola, CBS 281.96*.

76. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 12, or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10511, or

b) an analogue of the DNA sequence shown in SEQ ID No. 12 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10511, which

i) is homologous, preferably at least 60% homologous, with the DNA sequence shown in SEQ ID No. 12 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10511,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 12 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10511,

iii) encodes a polypeptide which is homologous, preferably at least 60% homologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 12 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10511,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 12 or obtainable from the plasmid in *Escherichia coli*, DSM 10511.

77. The DNA construct according to claim 76, in which the

DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family Tricholomataceae, preferably to the genus *Crinipellis,* in particular *Crinipellis scabella, especially C.scabella,* CBS 280.96.

78. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 16, or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10571, or

b) an analogue of the DNA sequence shown in SEQ ID No. 16 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10571, which

i) is homologous, preferably at least 70 % homologous, with the DNA sequence shown in SEQ ID No. 16 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10571,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 16 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10571,

iii) encodes a polypeptide which is homologous, preferably at least 60% hoologous, with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 16 or the DNA sequence obtainable from the plasmid in *Escherichia coli*, DSM 10571,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 16 or obtainable from the plasmid in *Escherichia coli*, DSM 10571.

79. The DNA construct according to claim 78, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain of *Volutella,* in particular *Volutella colletotrichoides, especially V. colletotrochoides, CBS 400.58.*

80. A DNA construct comprising a DNA sequence encoding an enzyme exhibiting endoglucanase activity, which DNA sequence comprises

a) the DNA sequence shown in SEQ ID No. 19, or the DNA sequence obtainable from the plasmid in *Escherichia coli,* DSM 10576, or

b) an analogue of the DNA sequence shown in SEQ ID No. 19 or the DNA sequence obtainable from the plasmid in *Escherichia coli,* DSM 10576, which

i) is homologous with the DNA sequence shown in SEQ ID No. 19 or the DNA sequence obtainable from the plasmid in *Escherichia coli,* DSM 10576,

ii) hybridizes under the conditions described herein with the same nucleotide probe as the DNA sequence shown in SEQ ID No. 19 or the DNA sequence obtainable from the plasmid in *Escherichia coli,* DSM 10576,

iii) encodes a polypeptide which is homologous with the polypeptide encoded by a DNA sequence comprising the DNA sequence shown in SEQ ID No. 19 or the DNA sequence obtainable from the plasmid in *Escherichia coli,* DSM 10576,

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified endoglucanase encoded by the DNA sequence shown in SEQ ID No 19 or obtainable from the plasmid in *Escherichia coli,* DSM 10576.

81. The DNA construct according to claim 80, in which the DNA sequence is isolated from or produced on the basis of a DNA library of a strain belonging to the family of *Sordariaceae*, preferably to the genus *Sordaria*, in particular *Sordaria fimicola*, especially S. fimicola, ATCC 52644.

82. The DNA construct according to any of the claims 66-81 which further comprises a DNA sequence encoding a cellulose-binding domain.

83. The DNA construct according to claim 82 which further comprises a DNA sequence encoding a cellulose-binding doain (CBD), the cellulose-binding domain and enzyme core (catalytically active domain) of the enzyme encoded by the DNA sequence of the DNA construct being operably linked.

84. A recombinant expression vector comprising a DNA construct according to any of claims 62-83.

85. A cell comprising a DNA construct according to any of claims 66-83 or a recombinant expression vector according to claim 84.

86. A cell according to claim 85, which is a eukaryotic cell, in particular a fungal cell, such as a yeast cell or a filamentous fungal cell, or an endogenous cell from which the gene originates.

87. A cell according to claim 86, wherein the cell belongs to a strain of *Aspergillus, Fusarium,* or *Trichoderma*, in particular a strain of *Fusarium graminearum, Aspergillus niger* or *Aspergillus oryzae*.

88. A method of producing an enzyme exhibiting endoglucanase activity, the method comprising culturing a

cell according to any of claims 85-87 under conditions permitting the production of the enzyme, and recovering the enzyme from the culture.

89. An enzyme exhibiting endoglucanase activity, which enzyme
a) is encoded by a DNA construct according to any of claims 66-83,
b) produced by the method according to claim 88, or
c) is immunologically reactive with an antibody raised against a purified endoglucanase encoded by the DNA sequence shown in any of the sequence listings SEQ ID No 1, 4, 6, 8, 10, 12, 16, 19.

90. A method of providing colour clarification of laundry, which method comprising treating the laundry with a soaking, washing or rinsing liquor comprising an enzyme preparation according to any of the claims 1-9, 11-61 and 89.

91. The method according to claim 90, wherein the laundry is treated in a washing machine.

92. The method according to claim 90 or 91, wherein the endoglucanase is present in the soaking, washing, or rinsing liquor in an effective amount of between 1 and 1000 S-CEVU, preferably between 5 and 200 S-CEVU, per liter of liquor during machine cycle use conditions.

93. The method according to any of the claim 90-92, wherein the pH of the soaking, washing, or rinsing liquor is between 4 and 11, preferably between 6 and 10.5.

94. The method according to any of the claims 90-93, whrein the temperature is between 15°C and 60°C.

95. The method according to any of the claims 90-94,

wherein the soaking, washing or rinsing liquor further comprises one or more enzymes selected from the group consisting of proteases, cellulases, xylanases, amylases, lipases, peroxidases and laccases.

96. A laundry composition comprising the enzyme preparation according to any of the claims 1-9, 11-61 and 89, and a compound selected from the group consisting of a surfactant, a builder compound, and a fabric softening agent.

97. The laundry composition according to claim 96, which further comprises one or more enzymes selected from the group consisting of proteases, amylases, lipases, cellulases, xylanases, peroxidases and laccases.

98. The composition according to claim 97, wherein the surfactant is a nonionic, anionic, cationic, zwitterionic, ampholytic or amphoteric surfactant.

99. The composition according to claim 98, wherein the fabric softening agent is a cationic or nonionic softening agent, preferably a quaternary ammonium compound, and which optionally further comprises one or more compounds selected from a surfactant, an electrolyte, a buffer, an antioxidant and a liquid carrier.

100. Use of the enzyme according to any of the claims 1-9, 11-61 and 89 for degradation or modification of plant material, e.g. cell walls.

101. Use of the enzyme according to any of the claims 1-9, 11-61 and 89 for treatment of fabric or textile, preferably for preventing backstaining, for bio-polishing or for "stone-washing" cellulosic fabric.

102. Use of the enzyme according to any of the claims 1-9, 11-61 and 89 in the treatment of paper pulp, preferably for debarking, defibration, fibre modification, enzymatic de-inking or drainage improvement.

103. An enzyme preparation which is enriched in an enzyme exhibiting cellulytic activity according to any of the claims 1-9, 11-61 and 89.

104. The preparation according to claim 103, which additionally comprises one or more enzymes selected from the group consisting of galactanases, xylanases, arabinanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, pectate lyases, endoglucanases, pectin methylesterases, proteases, lipases, amylases, cutinases, peroxidases, laccases, cellobiohydrolases and transglutaminases.

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Acremonium I               1  ------MRSTSILIGLVAGVAA---QSSGSGH  23
V. colletotrichoides       1  ------MRSSAVLIGLVAGVAA---QSSGTGR  23
C. scabella                1  MVHPNMLKTLAPLIILAASVTA---QTAGV--  27
Acremonium II              1  ------MISAWILLGLVGAVPSSVMAASGKGH  26
T. terrestris              1  ----MRSTPVLRTTLAAALPLVAS-AASGSGQ  27
M. thermophila             1  -------MHLSATTGFLALPVLALDQLSGIGQ  25
M. phaseolina              1  -----MFSPLWALSALLLFPAT----EATSGV  23

Acremonium I              24  TTRYWDCCKPSCAWDEKAAVSRPVTTCDRNNS  55
V. colletotrichoides      24  TTRYWDCCKPSCGWDEKASVSQPVKTCDRNNN  55
C. scabella               28  TTRYWDCCKPSCGWSGKASVSAPVRTCDRNGN  59
Acremonium II             27  TTRYWDCCKTSCAWEGKASVSEPVLTCNKQDN  58
T. terrestris             28  STRYWDCCKPSCAWPGKAAVSQPVYACDANFQ  59
M. thermophila            26  TTRYWDCCKPSCAWPGKGP-SSPVQACDKNDN  56
M. phaseolina             24  TTRYWDCCKPSCAWTGKASVSKPVGTCDINDN  55

Acremonium I              56  P-LSPG-AVSGCDPNGVAFTCNDNQPWAVNNN  85
V. colletotrichoides      56  P-LAST-ARSGCDSNGVAYTCNDNQPWAVNDN  85
C. scabella               60  T-LGPD-VKSGCDSGGTSFTCANNGPFAIDNN  89
Acremonium II             59  PIVDAN-ARSGCDGGG-AFACTNNSPWAVSED  88
T. terrestris             60  RLSDFN-VQSGCN-GGSAYSCADQTPWAVNDN  89
M. thermophila            57  PLNDGGSTRSGCDAGGSAYMCSSQSPWAVSDE  88
M. phaseolina             56  AQTPSDLLKSSCD-GGSAYYCSNQGPWAVNDS  86

Acremonium I              86  VAYGFAATAFPGGNEASWCCACYALQFTSGPV  117
V. colletotrichoides      86  LAYGFAATAFSGGSEASWCCACYALQFTSGPV  117
C. scabella               90  TAYGFAAAHLAGSSEAAWCCQCYELTFTSGPV  121
Acremonium II             89  LAYGFAATALSGGTEGSWCCACYAITFTSGPV  120
T. terrestris             90  LAYGFAATSIAGGSESSWCCACYALTFTSGPV  121
M. thermophila            89  LSYGWAAVKLAGSSESQWCCACYELTFTSGPV  120
M. phaseolina             87  LSYGFAAAKLSGKQETDWCCGCYKLTFTSTAV  118
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Fig. 1A

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Acremonium I           118  AGKTMVVQSTNTGGDLSGTHFDIQMPGGGLGI  149
V. colletotrichoides   118  AGKTMVVQSTNTGGDLSGNHFDILMPGGGLGI  149
C. scabella            122  VGKKLTVQVTNTGGDLGNNHFDLMIPGGGVGL  153
Acremonium II          121  AGKKMVVQSTNTGGDLSNNHFDLMIPGGGLGI  152
T. terrestris          122  AGKTMVVQSTSTGGDLGSNQFDIAMPGGGVGI  153
M. thermophila         121  AGKKMIVQATNTGGDLGDNHFDLAIPGGGVGI  152
M. phaseolina          119  SGKQMIVQITNTGGDLGNNHFDIAMPGGGVGI  150

Acremonium I           150  F-DGCTPQFGFTFP--GNRYGGTTSRSQCAEL  178
V. colletotrichoides   150  F-DGCTPQWGVSFP--GNRYGGTTSRSQCSQI  178
C. scabella            154  FTQGCPAQFGSWNG--GAQYGGVSSRDQCSQL  183
Acremonium II          153  F-DGCSAQFGQLLP--GERYGGVSSRSQCDGM  181
T. terrestris          154  F-NGCSSQFGGLP---GAQYGGISSRDQCDSF  181
M. thermophila         153  F-NACTDQYGAPPNGWGDRYGGIHSKEECESF  183
M. phaseolina          151  F-NGCSKQWNGI--NLGNQYGGFTDRSQCATL  179

Acremonium I           179  PSVLRDGCHWRYDWFNDADNPNVNWRRVRCPA  210
V. colletotrichoides   179  PSALQPGCNWRYDWFNDADNPDVSWRRVQCPA  210
C. scabella            184  PAAVQAGCQFRFDWMGGADNPNVTFRPVTCPA  215
Acremonium II          182  PELLKDGCQWRFDWFKNSDNPDIEFEQVQCPK  213
T. terrestris          182  PAPLKPGCQWRFDWFQNADNPTFTFQQVQCPA  213
M. thermophila         184  PEALKPGCNWRFDWFQNADNPSVTFQEVACPS  215
M. phaseolina          180  PSKWQASCNWRFDWFENADNPTVDWEPVTCPQ  211

Acremonium I           211  ALTNRSGCVRNDDNSYPVFEPGTGTPPTPTT-  241
V. colletotrichoides   211  ALTDRTGCRRSDDGNYPVFQPGPPPATTIRTS  242
C. scabella            216  QLTNISGCVRK---------------------  226
Acremonium II          214  ELIAVSGCVRDDDSSFPVFQGSGSGDVNPPPK  245
T. terrestris          214  EIVARSGCKRNDDSSFP-VFTPPSGGNGGTGT  244
M. thermophila         216  ELTSKSGCSR----------------------  225
M. phaseolina          212  ELVARTGCSRT---------------------  222
```

Fig. 1B

```
Acremonium I          242  --------------------------------  241
V. colletotrichoides  243  TTITASSSSSSSSSS-----------------  257
C. scabella             0  --------------------------------  226
Acremonium II         246  PTTTTTSSKPKTTSAPSTLSNPSAPQQPGNTD  277
T. terrestris         245  PTSTAPGSGQ----------------------  254
M. thermophila          0  --------------------------------  225
M. phaseolina           0  --------------------------------  222

Acremonium I          242  -------------TTTSSPPQPTNGGGGGTSP  260
V. colletotrichoides  258  -------------TTAGSPPVPTGGGSGPTSP  276
C. scabella             0  --------------------------------  226
Acremonium II         278  RPAETTTTKLPALPATTSSPAVSVPSSSARVP  309
T. terrestris         255  --------------TS------PGGGSGCTSQ  266
M. thermophila          0  --------------------------------  225
M. phaseolina           0  --------------------------------  222

Acremonium I          261  HWGQCGGQG-WSGPTACAGGSTCNLINPWYSQ  291
V. colletotrichoides  277  VWGQCGGQG-WSGPTRCVAGSTCSVVNPWYSQ  307
C. scabella             0  --------------------------------  226
Acremonium II         310  LWGQCDSEASWDAPKKCAKGTKCVYVNDWYSQ  341
T. terrestris         267  KWAQCGGIG-FSGCTTCVSGTTCQKLNDYYSQ  297
M. thermophila          0  --------------------------------  225
M. phaseolina           0  --------------------------------  222

Acremonium I          292  CIPN----------------------------  295
V. colletotrichoides  308  CFP-----------------------------  310
C. scabella             0  --------------------------------  226
Acremonium II         342  CQPKNSCA------------------------  349
T. terrestris         298  CL------------------------------  299
M. thermophila          0  --------------------------------  225
M. phaseolina           0  --------------------------------  222
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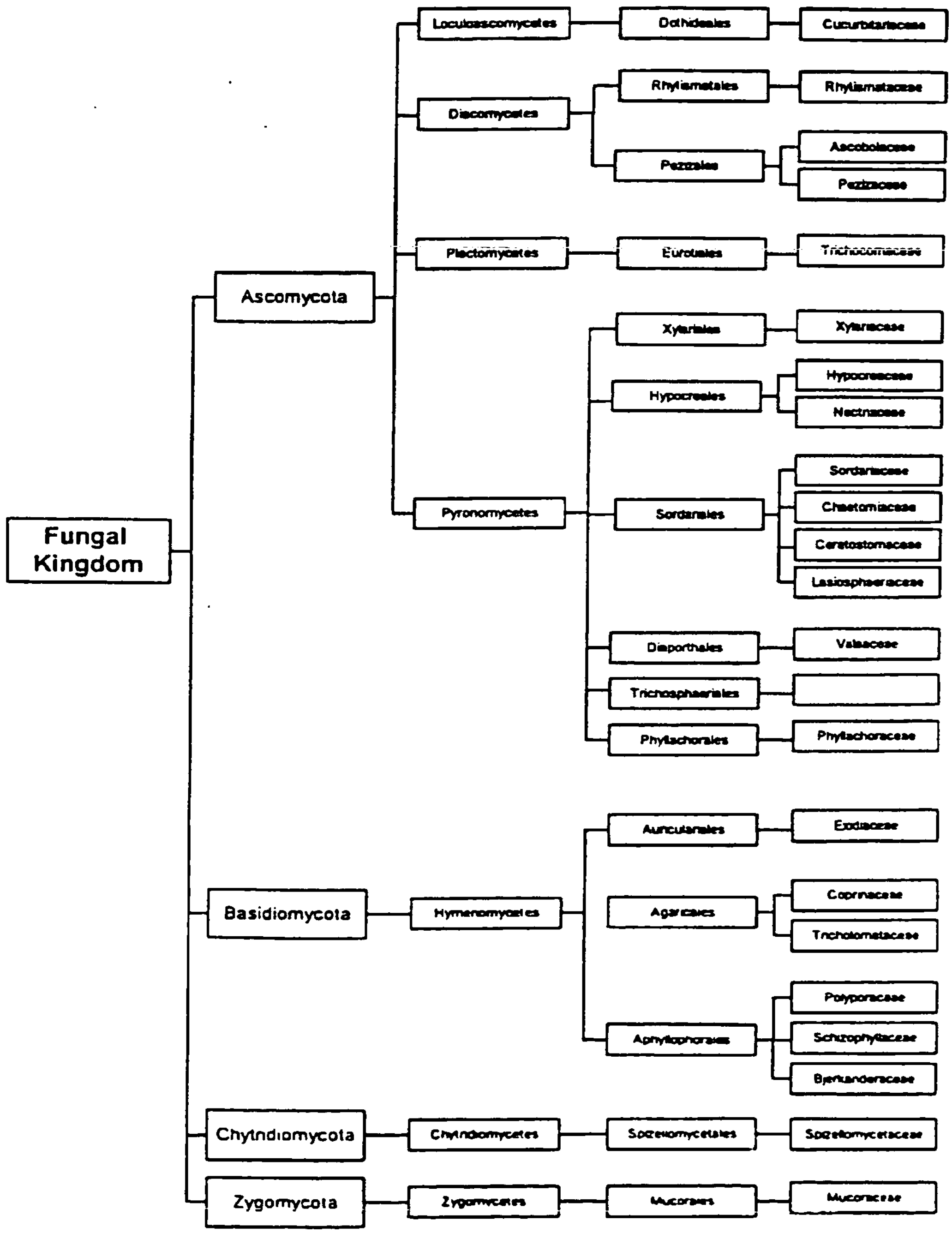
Fig. 1C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Ascobolaceae
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Eurotiales
Trichocomaceae
Pyrenomycetes
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Ceratostomaceae
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Agaricales
Coprinaceae
Tricholomataceae
Aphyllophorales
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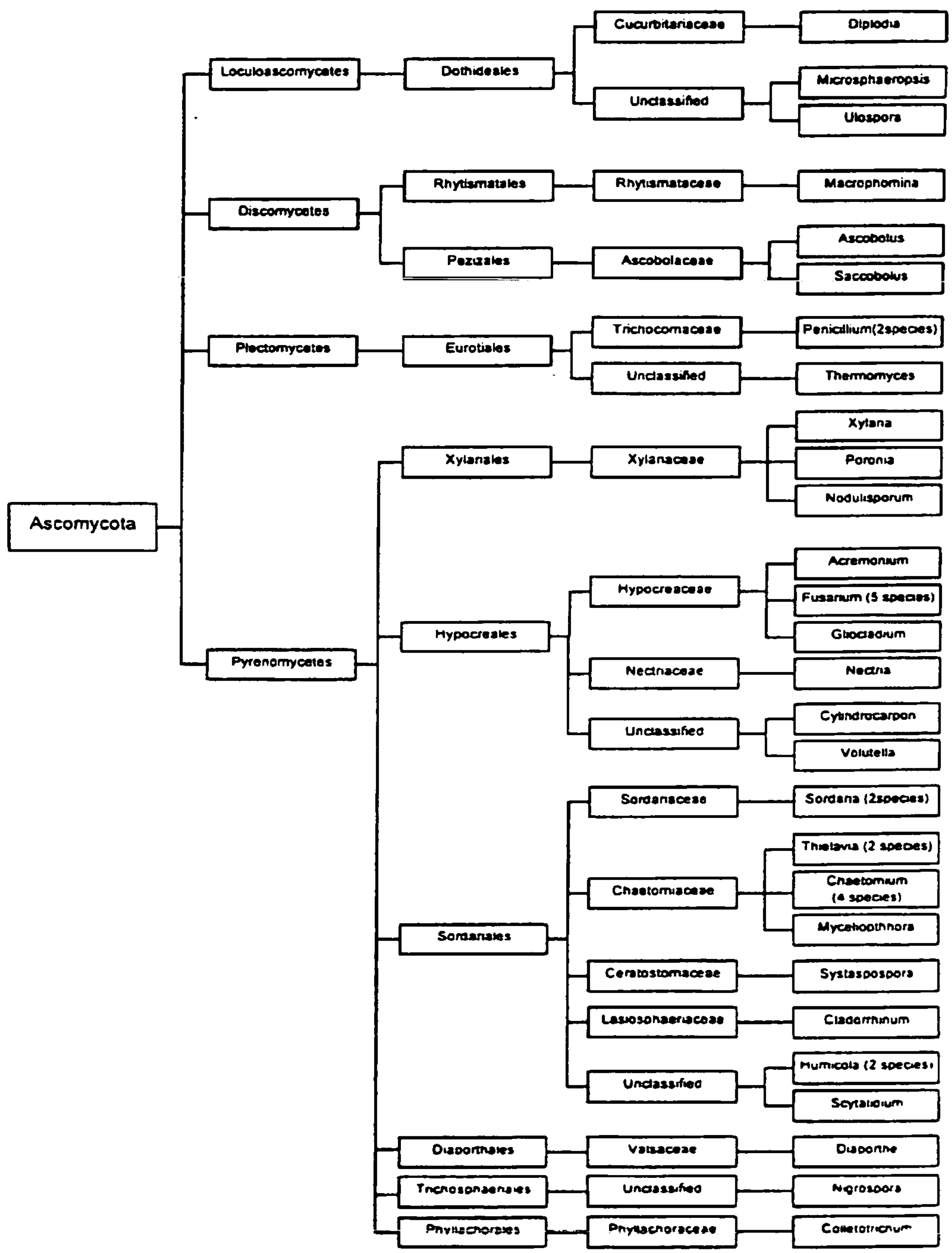
Mucoraceae

Fig. 2A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Macrophomina
Pezizales
Ascobolaceae
Ascobolus
Saccobolus
Plectomycetes
Eurotiales
Trichocomaceae
Penicillium(2species)
Unclassified
Thermomyces
Pyrenomycetes
Xylariales
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Poronia
Nodulisporum
Hypocreales
Hypocreaceae
Acremonium
Fusarium (5 species)
Gliocladium
Nectriaceae
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Unclassified
Cylindrocarpon
Volutella
Sordariales
Sordariaceae
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Chaetomiaceae
Thielavia (2 species)
Chaetomium (4 species)
Myceliophthora
Ceratostomaceae
Systasospora
Lasiosphaeriaceae
Cladorrhinum
Unclassified
Humicola (2 species)
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Diaporthales
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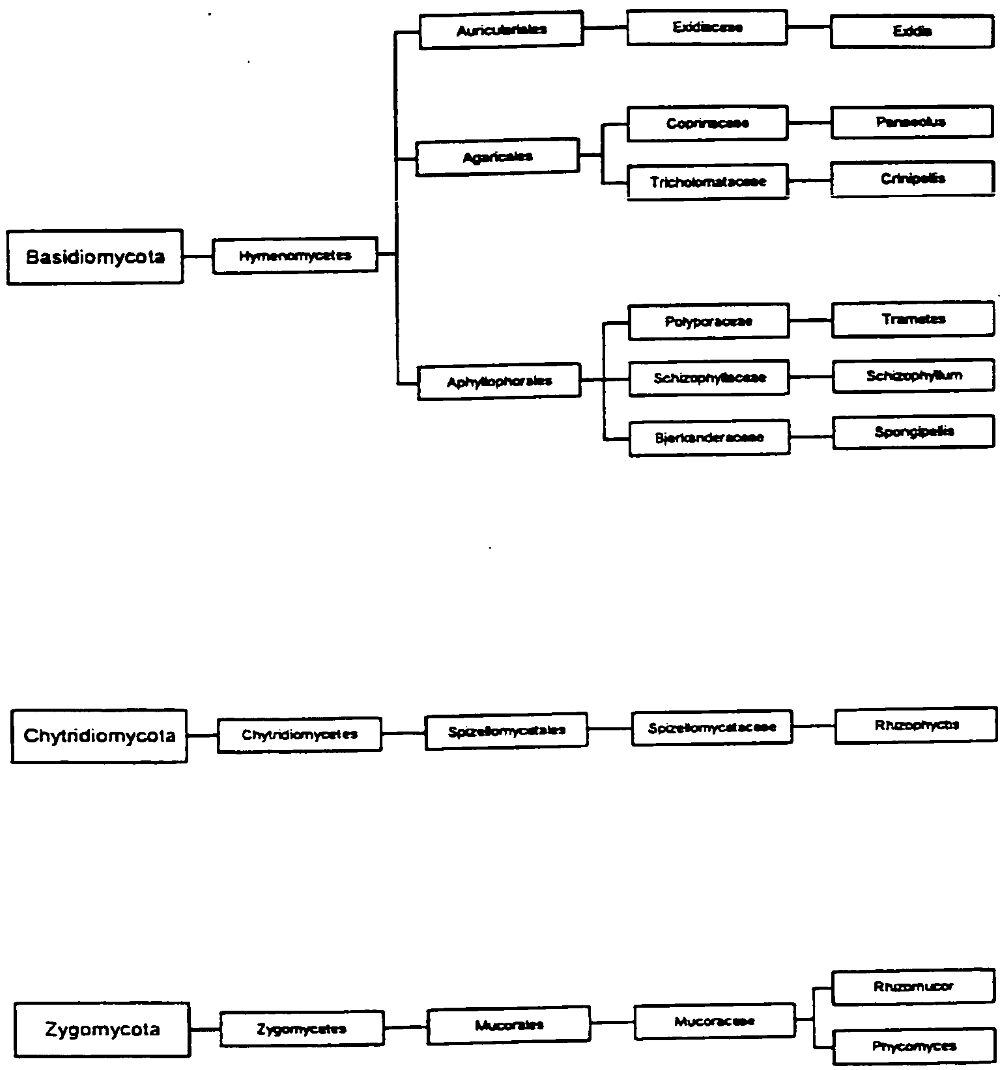
Colletotrichum

Fig. 2B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Polyporaceae
Trametes
Schizophyllaceae
Schizophyllum
Bjerkanderaceae
Spongipellis
Chytridiomycota
Chytridiomycetes
Spizellomycetales
Spizellomycetaceae
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Zygomycetes
Mucorales
Mucoraceae
Rhizomucor
Phycomyces

Fig. 2C

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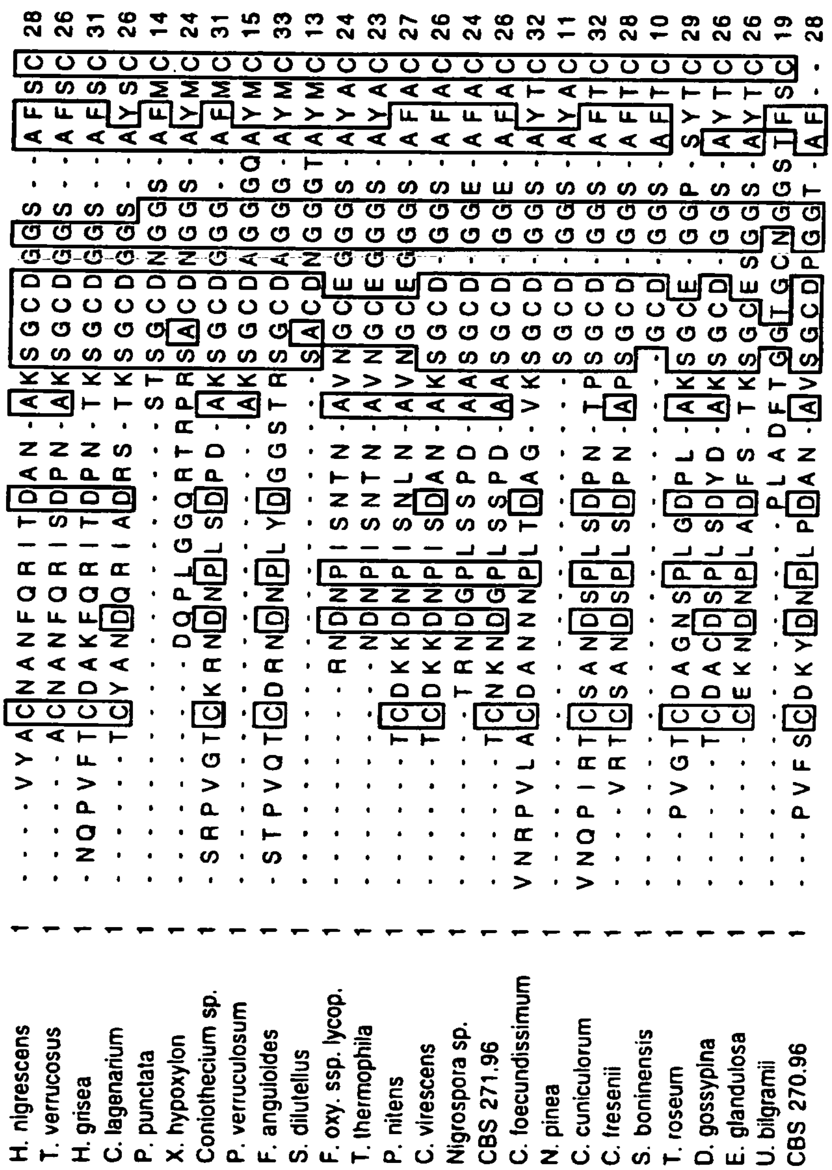
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T. verrucosus
H. grisea
C. lagenarium
P. punctata
X. hypoxylon
Coniothecium sp.
P. verruculosum
F. anguioides
S. dilutellus
F. oxy. ssp. lycop.
T. thermophila
P. nitens
C. virescens
Nigrospora sp.
CBS 271.96
C. foecundissimum
N. pinea
C. cuniculorum
C. fresenii
S. boninensis
T. roseum
D. gossypina
E. glandulosa
U. bilgramii
CBS 270.96

Fig. 3A

```
H. nigrescens          29  ANQTPWAVSDDFAYGFAATALAG------------  51
T. verrucosus          27  AKQTPWA----------------------------  33
H. grisea              32  ADQTPWALNDDFAYGFAATAISGGSEAS-------  59
C. lagenarium          27  S----------------------------------  27
P. punctata            15  SNQSPWAVNDDLAYGWAAVSIAG------------  37
X. hypoxylon           25  SNQSPWAVDDSLSYGWAAVRIYGHTETTWCCACYE  59
Coniothecium sp.       32  STQQPWAVNDNLAYGFAATAISGGNE---------  57
P. verruculosum        16  SNQQPWVVNDNLAYGFAAVNIAG------------  38
F. anguioides          34  SSHSPWAVSDSLSYGWAAVRIAGQSE---------  59
S. dilutellus          14  ASQEPWAVSSNVAYGFAAVRISG------------  36
F. oxy. ssp. lycop.    25  TNYSPWAVNDELAYGFAATKISGGSEASWCCACYL  59
T. thermophila         24  SNYSPWAVNDDLAYGFAVTKISGGSEA--------  50
P. nitens              28  TNYSPWAVNDNLAYGFAATKLAGGSE---------  53
C. virescens           27  TNYSPFAVNDNLAYGFAATKLAGGSEA--------  53
Nigrospora sp.         25  SNTSPWAVSDQLAYGYVATSISGGTES--------  51
CBS 271.96             27  SNTSPWAVSDQLAYGYLATSISGGTES--------  53
C. foecundissimum      33  ANNSPWAVNDQLAYGFAATKLSGGTESS-------  60
N. pinea               12  ANNSPWAVND-------------------------  21
C. cuniculorum         33  SNNSPWAVDDQTAYGFAATAISGQSESS-------  60
C. fresenii            29  SNNSPWAVDDQTAYGFAATAISGQS----------  53
S. boninensis          11  SNNSPWAVNED------------------------  21
T. roseum              30  ANYQPWAVNDQLAYGFAATAINGGTE---------  55
D. gossypina           27  TYSTPWAVDDNLSYGFAAAKLSG------------  49
E. glandulosa          27  NNQSPWAVNDLVSYGFAATAINGGN----------  51
U. bilgramii           20  SNQQPWAVNDTFSYGFAGIFITGHVE---------  45
CBS 270.96              0  -----------------------------------  28
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Fig. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00105

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/42, C11D 3/386, D06M 16/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, EMBL/GENBANK/DDBJ/SWISSPROT (STRAND)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9407998 A1 (NOVO NORDISK A/S), 14 April 1994 (14.04.94), see fig 1A --	1-104
A	EP 0508358 A1 (THE PROCTER & GAMBLE COMPANY), 14 October 1992 (14.10.92) --	1-104
A	EP 0495257 A1 (THE PROCTER & GAMBLE COMPANY), 22 July 1992 (22.07.92) --	1-104
A	WO 9117243 A1 (NOVO NORDISK A/S), 14 November 1991 (14.11.91) --	1-104

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 June 1996	02-07-1996

Name and mailing address of the ISA/	Authorized officer
Sw dish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Yvonne Siösteen Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00105

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9320193 A1 (NOVO NORDISK A/S), 14 October 1993 (14.10.93) --	1-104
A	WO 9502043 A1 (NOVO NORDISK A/S), 19 January 1995 (19.01.95) --	1-104
A	Eur. J. Biochem., Volume 146, 1985, Gerrit Beldman et al, "The cellulase of Trichoderma viride" page 301 - page 308 -- --------	1-104

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/96

International application No.
PCT/DK 96/00105

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		WO-A,A- 9213054	06/08/92
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Information on patent family members

01/04/96

International application No.
PCT/DK 96/00105

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		AU-A- 7887491	27/11/91
		CA-A- 2082279	10/11/91
		DE-D,T- 69107455	12/10/95
		EP-A,A,B 0531372	17/03/93
		ES-T- 2068586	16/04/95
		JP-T- 5509223	22/12/93
WO-A1- 9320193	14/10/93	NONE	
WO-A1- 9502043	19/01/95	NONE	